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(71) Applicant: CORIXA CORPORATION [US/US]; Suite 200, 1124 Columbia Street, Seattle, WA 98104 (US).

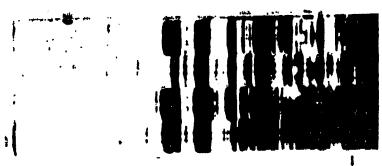
(72) Inventors: FRUDAKIS, Tony, N.; 7937 Broadmoor Pines Boulevard, Sarasota, FL 34243 (US). SMITH, John, M.; 208 – 116th Place S.E., Everett, WA 98208 (US). REED, Steven, G.; 2843 – 122nd Place N.E., Bellevue, WA 98005 (US). MISHER, Lynda, E.; 6251 53rd Avenue N.E., Seattle, WA 98115 (US). RETTER, Marc, W.; 33402 N.E. 43rd Place, Carnation, WA 98014 (US). DILLON, Davin, C.; 21607 N.E. 24th Street, Redmond, WA 98053 (US).

(74) Agents: POTTER, Jane, E., R.; Seed Intellectual Property Law Group PLLC, Suite 6300, 701 Fifth Avenue, Seattle, WA 98104-7092 (US) et al. (81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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CDNA PREPARED FROM NORMAL BREAST TISSUE FROM THE SAME PATIENT

cDNA PREPARED FROM BREAST TUMOR

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(57) Abstract

Compositions and methods for the detection and therapy of breast cancer are disclosed. The compounds provided include nucleotide sequences that are preferentially expressed in breast tumor tissue, as well as polypeptides encoded by such nucleotide sequences. Vaccines and pharmaceutical compositions comprising such compounds are also provided and may be used, for example, for the prevention and treatment of breast cancer. The polypeptides may also be used for the production of antibodies, which are useful for diagnosing and monitoring the progression of breast cancer in a patient.

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COMPOSITIONS AND METHODS FOR THE TREATMENT AND DIAGNOSIS OF BREAST CANCER

TECHNICAL FIELD

The present invention relates generally to the detection and therapy of breast cancer. The invention is more specifically related to nucleotide sequences that are preferentially expressed in breast tumor tissue and to polypeptides encoded by such nucleotide sequences. The nucleotide sequences and polypeptides may be used in vaccines and pharmaceutical compositions for the prevention and treatment of breast cancer. The polypeptides may also be used for the production of compounds, such as antibodies, useful for diagnosing and monitoring the progression of breast cancer in a patient.

BACKGROUND OF THE INVENTION

Breast cancer is a significant health problem for women in the United States and throughout the world. Although advances have been made in detection and treatment of the disease, breast cancer remains the second leading cause of cancer-related deaths in women, affecting more than 180,000 women in the United States each year. For women in North America, the life-time odds of getting breast cancer are now one in eight.

No vaccine or other universally successful method for the prevention or treatment of breast cancer is currently available. Management of the disease currently relies on a combination of early diagnosis (through routine breast screening procedures) and aggressive treatment, which may include one or more of a variety of treatments such as surgery, radiotherapy, chemotherapy and hormone therapy. The course of treatment for a particular breast cancer is often selected based on a variety of prognostic parameters, including an analysis of specific tumor markers. See, e.g., Porter-Jordan and Lippman, Breast Cancer 8:73-100 (1994). However, the use of established markers often leads to a result that is difficult to interpret, and the high mortality observed in

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breast cancer patients indicates that improvements are needed in the treatment, diagnosis and prevention of the disease.

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Accordingly, there is a need in the art for improved methods for therapy and diagnosis of breast cancer. The present invention fulfills these needs and further provides other related advantages.

SUMMARY OF THE INVENTION

Briefly stated, the subject invention provides compositions and methods for the diagnosis and therapy of breast cancer. In one aspect, isolated polynucleotides are provided, comprising (a) a nucleotide sequence preferentially expressed in breast cancer tissue, relative to normal tissue; (b) a variant of such a sequence, as defined below; or (c) a nucleotide sequence encoding an epitope of a polypeptide encoded by at least one of the above sequences. In one embodiment, the isolated polynucleotide comprises a human endogenous retroviral sequence recited in SEQ ID NO:1. In other embodiments, the isolated polynucleotide comprises a sequence recited in any one of SEQ ID NO: 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317.

In related embodiments, the isolated polynucleotide encodes an epitope of a polypeptide, wherein the polypeptide is encoded by a nucleotide sequence that: (a) hybridizes to a sequence recited in any one of SEQ ID NO: 1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317 under stringent conditions; and (b) is at least 80% identical to a sequence recited in any one of SEQ ID NO: 1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317.

 $z_1 = \{ z_1, \ldots, z_n \}$

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In another embodiment, the present invention provides an isolated polynucleotide encoding an epitope of a polypeptide, the polypeptide being encoded by: (a) a nucleotide sequence transcribed from the sequence of SEQ ID NO: 141; or (b) a variant of said nucleotide sequence that contains one or more nucleotide substitutions, deletions, insertions and/or modifications at no more than 20% of the nucleotide positions, such that the antigenic and/or immunogenic properties of the polypeptide encoded by the nucleotide sequence are retained. Isolated DNA and RNA molecules comprising a nucleotide sequence complementary to a polynucleotide as described above are also provided. The secure of the lapter to the secure Commenced Green Street Broken Broken

In related aspects, the present invention provides recombinant expression vectors comprising a polynucleotide as described above and host cells transformed or transfected with such expression vectors, and the such expression vectors and the such expression vectors and the such expression vectors.

In further aspects, polypeptides comprising an amino acid sequence encoded by a polynaricotide as described above, and monoclonal antibodies that bind to such polypeptides are rovided. In certain embodiments, the inventive polypeptides comprise an amino acid sequence selected from the group consisting of SEQ ID NO: 299, 300, 304-306, 308 and 315, and variants thereof as defined below.

ben dit with the In yet another aspect, methods are provided for determining the presence of breast cancer in a patient. In one embodiment, the method comprises detecting, within 20 a biological sample, a polypeptide as described above. In another embodiment, the method comprises detecting, within a biological sample, an RNA molecule encoding a polypeptide as described above. In yet another embodiment, the method comprises (a) intradermally injecting a patient with a polypeptide as described above; and (b) detecting an immune response on the patient's skin and therefrom detecting the presence of breast 25 cancer in the patient. In further embodiments, the present invention provides methods for determining the presence of breast cancer in a patient as described above wherein the polypeptide is encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 78486, 144, 145, 153, 167, 177, 193, 199, 205, 208, 215, 217, 220, 241, 242, 246, 248, 249, 252, 256, 267, 270, 274, 277, 279, 282, 283, 285-287, 289, 290 and sequences that hybridize thereto under stringent conditions.

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In a related aspect, diagnostic kits useful in the determination of breast cancer are provided. The diagnostic kits generally comprise either one or more monoclonal antibodies as described above, or one or more monoclonal antibodies that bind to a polypeptide encoded by a nucleotide sequence selected from the group consisting of sequences provided in SEQ ID NO: 78-86, 144, 145, 153, 167, 177, 193, 199, 205, 208, 215, 217, 220, 241, 242 and 246, 248, 249, 252, 256, 267, 270, 274, 277, 279, 282, 283, 285-287, 289, 290 and a detection reagent.

Diagnostic kits are also provided that comprise a first polymerase chain reaction primer and a second polymerase chain reaction primer, at least one of the primers being specific for a polynucleotide described herein. In one embodiment, at least one of the primers comprises at least about 10 contiguous nucleotides of a polynucleotide as described above, or a polynucleotide encoding a polypeptide encoded by a sequence selected from the group consisting of SEQ ID NO: 78-86, 144, 145, 153, 167, 177, 193, 199, 205, 208, 215, 217, 220, 241, 242 246, 248, 249, 252, 256, 267, 270, 274, 277, 279, 282, 283, 285-287, 289 and 290.

Within another related aspect, the diagnostic kit comprises at least one oligonucleotide probe, the probe being specific for a polynucleotide described herein. In one embodiment, the probe comprises at least about 15 contiguous nucleotides of a polynucleotide as described above, or a polynucleotide selected from the group consisting of SEQ ID NO: 78-86, 144, 145, 153, 167, 177, 193, 199, 205, 208, 215, 217, 220, 241, 242 246, 248, 249, 252, 256, 267, 270, 274, 277, 279, 282, 283, 285-287, 289 and 290.

In another related aspect, the present invention provides methods for monitoring the progression of breast cancer in a patient. In one embodiment, the method comprises: (a) detecting an amount, in a biological sample, of a polypeptide as described above at a first point in time; (b) repeating step (a) at a subsequent point in time; and (c) comparing the amounts of polypeptide detected in steps (a) and (b), and therefrom monitoring the progression of breast cancer in the patient. In another embodiment, the method comprises (a) detecting an amount, within a biological sample, of an RNA molecule encoding a polypeptide as described above at a first point in time; (b) repeating

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step (a) at a subsequent point in time; and (c) comparing the amounts of RNA molecules detected in steps (a) and (b), and therefrom monitoring the progression of breast cancer in the patient. In yet other embodiments, the present invention provides methods for monitoring the progression of breast cancer in a patient as described above wherein the polypeptide is encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 78-86, 144, 145, 153, 167, 177, 193, 199, 205, 208, 215, 217, 220, 241, 242, 246, 248, 249, 252, 256, 267, 270, 274, 277, 279, 282, 283, 285-287, 289, 290 and sequences that hybridize thereto under stringent conditions.

In still other aspects, pharmaceutical compositions, which comprise a polypeptide as described above in combination with a physiologically acceptable carrier, and vaccines, which comprise a polypeptide as described above in combination with an immunostimulant or adjuvant, are provided. In yet other aspects, the present invention provides pharmaceutical compositions and vaccines comprising a polypeptide encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 78-86, 144, 145, 153, 167, 177, 193, 199, 205, 208, 215, 217, 220, 241, 242 and 246, 248, 249, 252, 256, 267, 270, 274, 277, 279, 282, 283, 285-287, 289, 290 and sequences that hybridize thereto under stringent conditions.

In related aspects, the present invention provides methods for inhibiting the development of breast cancer in a patient, comprising administering to a patient a pharmaceutical composition or vaccine as described above.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS

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Figure 1 shows the differential display PCR products, separated by gel electrophoresis, obtained from cDNA prepared from normal breast tissue (lanes 1 and 2) and from cDNA prepared from breast tumor tissue from the same patient (lanes 3 and 4). The arrow indicates the band corresponding to B18Ag1.

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Figure 2 is a northern blot comparing the level of B18Ag1 mRNA in breast tumor tissue (lane 1) with the level in normal breast tissue.

Figure 3 shows the level of B18Ag1 mRNA in breast tumor tissue compared to that in various normal and non-breast tumor tissues as determined by RNase protection assays.

Figure 4 is a genomic clone map showing the location of additional retroviral sequences obtained from ends of XbaI restriction digests (provided in SEQ ID NO:3 - SEQ ID NO:10) relative to B18Ag1.

Figures 5A and 5B show the sequencing strategy, genomic organization and predicted open reading frame for the retroviral element containing B18Ag1.

Figure 6 shows the nucleotide sequence of the representative breast tumor-specific cDNA B18Ag1.

Figure 7 shows the nucleotide sequence of the representative breast tumor-specific cDNA B17Ag1.

Figure 8 shows the nucleotide sequence of the representative breast tumor-specific cDNA B17Ag2.

Figure 9 shows the nucleotide sequence of the representative breast tumor-specific cDNA B13Ag2a

Figure 10 shows the nucleotide sequence of the representative breast tumor-specific cDNA B13Ag1b.

Figure 11 shows the nucleotide sequence of the representative breast tumor-specific cDNA B13Ag1a

Figure 12 shows the nucleotide sequence of the representative breast tumor-specific cDNA B11Ag1.

Figure 13 shows the nucleotide sequence of the representative breast tumor-specific cDNA B3CA3c.

Figure 14 shows the nucleotide sequence of the representative breast tumor-specific cDNA B9CG1.

Figure 15 shows the nucleotide sequence of the representative breast tumor-specific cDNA B9CG3.

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Figure 16 shows the nucleotide sequence of the representative breast tumor-specific cDNA B2CA2.

Figure 17 shows the nucleotide sequence of the representative breast tumor-specific cDNA B3CA1.

Figure 18 shows the nucleotide sequence of the representative breast tumor-specific cDNA B3CA2.

Figure 19 shows the nucleotide sequence of the representative breast tumor-specific cDNA B3CA3.

Figure 20 shows the nucleotide sequence of the representative breast tumor-specific cDNA B4CA1.

Figure 21A depicts RT-PCR analysis of breast tumor genes in breast tumor tissues (lanes 1-8) and normal breast tissues (lanes 9-13) and H₂O (lane 14).

Figure 21B depicts RT-PCR analysis of breast tumor genes in prostate tumors (lane 1, 2), colon tumors (lane 3), lung tumor (lane 4), normal prostate (lane 5), normal colon (lane 6), normal kidney (lane 7), normal liver (lane 8), normal lung (lane 9), normal ovary (lanes 10, 18), normal pancreases (lanes 11, 12), normal skeletal muscle (lane 13), normal skin (lane 14), normal stomach (lane 15), normal testes (lane 16), normal small intestine (lane 17), HBL-100 (lane 19), MCF-12A (lane 20), breast tumors (lanes 21-23), H₂O (lane 24), and colon tumor (lane 25).

Figure 22 shows the recognition of a B11Ag1 peptide (referred to as B11-8) by an anti-B11-8 CTL line.

Figure 23 shows the recognition of a cell line transduced with the antigen B11Ag1 by the B11-8 specific clone A1.

Figure 24 shows recognition of a lung adenocarcinoma line (LT-140-22) and a breast adenocarcinoma line (CAMA-1) by the B11-8 specific clone A1.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to compositions and methods for the diagnosis, monitoring and therapy of breast cancer. The compositions described herein include polypeptides, polynucleotides and antibodies.

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Polypeptides of the present invention generally comprise at least a portion of a protein that is expressed at a greater level in human breast tumor tissue than in normal breast tissue (i.e., the level of RNA encoding the polypeptide is at least 2-fold higher in tumor tissue). Such polypeptides are referred to herein as breast tumor-specific polypeptides, and cDNA molecules encoding such polypeptides are referred to as breast tumor-specific cDNAs. Polynucleotides of the subject invention generally comprise a DNA or RNA sequence that encodes all or a portion of a polypeptide as described above, or that is complementary to such a sequence. Antibodies are generally immune system proteins, or fragments thereof, that are capable of binding to a portion of a polypeptide as described above. Antibodies can be produced by cell culture techniques, including the generation of monoclonal antibodies as described herein, or via transfection of antibody genes into suitable bacterial or mammalian cell hosts, in order to allow for the production of recombinant antibodies.

Polypeptides within the scope of this invention include, but are not limited to, polypeptides (and epitopes thereof) encoded by a human endogenous retroviral sequence, such as the sequence designated B18Ag1 (Figure 5 and SEQ ID NO:1). Also within the scope of the present invention are polypeptides encoded by other sequences within the retroviral genome containing B18Ag1 (SEQ ID NO: 141). Such sequences include, but are not limited to, the sequences recited in SEQ ID NO:3 - SEQ ID NO:10. B18Ag1 has homology to the gag p30 gene of the endogenous human retroviral element S71, as described in Werner et al., Virology 174:225-238 (1990) and also shows homology to about thirty other retroviral gag genes. As discussed in more detail below, the present invention also includes a number of additional breast tumor-specific polypeptides, such as those encoded by the nucleotide sequences recited in SEQ ID NO: 11-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317.

As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins containing the sequences recited herein. A

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polypeptide comprising an epitope of a protein containing a sequence as described herein may consist entirely of the epitope, or may contain additional sequences. The additional sequences may be derived from the native protein or may be heterologous, and such sequences may (but need not) possess immunogenic or antigenic properties.

An "epitope," as used herein is a portion of a polypeptide that is recognized (i.e., specifically bound) by a B-cell and/or T-cell, surface antigen receptor. Epitopes may generally be identified using well known techniques, such as those summarized in Paul, Fundamental Immunology, 3rd ed., 243-247 (Raven Press, 1993) and references cited therein. Such techniques include screening polypeptides derived from the native polypeptide for the ability to react with antigen-specific antisera and/or T-cell lines or clones. An epitope of a polypeptide is a portion that reacts with such antisera and/or T-cells at a level that is similar to the reactivity of the full length polypeptide (e.g., in an ELISA and/or T-cell reactivity assay). Such screens may generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988. B-cell and T-cell epitopes may also be predicted via computer analysis. Polypeptides comprising an epitope of a polypeptide that is preferentially expressed in a tumor tissue (with or without additional amino acid sequence) are within the scope of the present invention.

The term "polynucleotide(s)," as used herein, means a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases and includes DNA and corresponding RNA molecules, including HnRNA and mRNA molecules, both sense and anti-sense strands, and comprehends cDNA, genomic DNA and recombinant DNA, as well as wholly or partially synthesized polynucleotides. An HnRNA molecule contains introns and corresponds to a DNA molecule in a generally one-to-one manner. An mRNA molecule corresponds to an HnRNA and DNA molecule from which the introns have been excised. A polynucleotide may consist of an entire gene, or any portion thereof. Operable anti-sense polynucleotides may comprise a fragment of the corresponding polynucleotide, and the definition of "polynucleotide" therefore includes all such operable anti-sense fragments.

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The compositions and methods of the present invention also encompass variants of the above polypeptides and polynucleotides.

A polypeptide "variant," as used herein, is a polypeptide that differs from the recited polypeptide only in conservative substitutions and/or modifications, such that the antigenic properties of the polypeptide are retained. In a preferred embodiment, variant polypeptides differ from an identified sequence by substitution, deletion or addition of five amino acids or fewer. Such variants may generally be identified by modifying one of the above polypeptide sequences, and evaluating the antigenic properties of the modified polypeptide using, for example, the representative procedures described herein. Polypeptide variants preferably exhibit at least about 70%, more preferably at least about 90% and most preferably at least about 95% identity (determined as described below) to the identified polypeptides.

As used herein, a "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. In general, the following groups of amino acids represent conservative changes: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his.

Variants may also, or alternatively, contain other modifications, including the deletion or addition of amino acids that have minimal influence on the antigenic properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

A nucleotide "variant" is a sequence that differs from the recited nucleotide sequence in having one or more nucleotide deletions, substitutions or

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additions. Such modifications may be readily introduced using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis as taught, for example, by Adelman et al. (DNA, 2:183, 1983). Nucleotide variants may be naturally occurring allelic variants, or non-naturally occurring variants. Variant nucleotide sequences preferably exhibit at least about 70%, more preferably at least about 80% and most preferably at least about 90% identity (determined as described below) to the recited sequence.

The breast tumor antigens provided by the present invention include variants that are encoded by DNA sequences which are substantially homologous to one or more of the DNA sequences specifically recited herein. "Substantial homology," as used herein, refers to DNA sequences that are capable of hybridizing under moderately stringent conditions. Suitable moderately stringent conditions include prewashing in a solution of 5X/SSC, 0.5% SDS, 1.0 and EDTA (pH 8.0); hybridizing at 50°C-65°C, 5X SSC, overnight or, in the event of cross-species homology, at 45°C with 0.5X SSC; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS. Such hybridizing DNA sequences are also within the scope of this invention, as are nucleotide sequences that, due to code degeneracy, encode an immunogenic polypeptide that is encoded by a hybridizing DNA sequence.

Two nucleotide or polypeptide sequences are said to be "identical" if the sequence of nucleotides or amino acid residues in the two sequences is the same when aligned for maximum correspondence as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment

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from suitable host/vector systems which secrete recombinant protein or polypeptide into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant polypeptide.

In general, any of a variety of expression vectors known to those of ordinary skill in the art may be employed to express recombinant polypeptides of this invention. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a polynucleotide that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line such as COS or CHO.

Such techniques may also be used to prepare polypeptides comprising epitopes or variants of the native polypeptides. For example, variants of a native polypeptide may generally be prepared using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis, and sections of the DNA sequence may be removed to permit preparation of truncated polypeptides. Portions and other variants having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may also be generated by synthetic means, using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. See Merrifield, J. Am. Chem., Soc. 85:2149-2146 (1963). Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied BioSystems Division,, Foster, City, CA, and may be operated according to the manufacturer's instructions.

In specific embodiments, polypeptides of the present invention encompass amino acid sequences encoded by a polynucleotide having a sequence recited in any one of SEQ ID NO:1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255,

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257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317, and variants of such polypeptides. Polypeptides within the scope of the present invention also include polypeptides (and epitopes thereof) encoded by DNA sequences that hybridize to a, sequence recited in any one of SEQ ID NO:1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317 under stringent conditions, wherein the DNA sequences are at least 80% identical in overall sequence to a recited sequence and wherein RNA corresponding to the nucleotide sequence is expressed at a greater level in human breast tumor tissue than in normal breast tissue. As used herein, "stringent conditions" refers to prewashing in a solution of 6X SSC, 0.2% SDS; hybridizing at 65°C, 6X SSC, 0.2% SDS overnight; followed by two washes of 30 minutes each in 1X SSC, 0.1% SDS at 65°C and two washes of 30 minutes each in 0.2 X SSC, 0.1% SDS at 65°C. Polynucleotides according to the present invention include molecules that encode any of the above polypeptides.

In another aspect of the present invention, antibodies are provided. Such antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. See, e.g., Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Haroor Laboratory, 1988. In one such technique, an immunogen comprising the polypeptide is initially injected into any of a wide variety of mammals (e.g., mice, rats, rabbits, sheep or goats). In this step, the polypeptides of this invention may serve as the immunogen without modification. Aiternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

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Monoclonal antibodies specific for the antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, Eur. J. Immunol. 6:511-519 (1976), and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (i.e., reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and their culture supernatants tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. The polypeptides of this invention may be used in the purification process in, for example, an affinity chromatography step.

Antibodies may be used, for example, in methods for detecting breast cancer in a patient. Such methods involve using an antibody to detect the presence or absence of a breast tumor-specific polypeptide as described herein in a suitable biological sample. As used herein, suitable biological samples include tumor or normal tissue biopsy, mastectomy, blood, lymph node, serum or urine samples, or other tissue, homogenate, or extract thereof obtained from a patient.

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There are a variety of assay formats known to those of ordinary skill in the art for using an antibody to detect polypeptide markers in a sample. See, e.g., Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988. For example, the assay may be performed in a Western blot format, wherein a protein preparation from the biological sample is submitted to gel electrophoresis, transferred to a suitable membrane and allowed to react with the antibody. The presence of the antibody on the membrane may then be detected using a suitable detection reagent, as described below.

In another embodiment, the assay involves the use of antibody immobilized on a solid support to bind to the polypeptide and remove it from the remainder of the sample. The bound polypeptide may then be detected using a second antibody or reagent that contains a reporter group. Alternatively, a competitive assay may be utilized, in which a polypeptide is labeled with a reporter group and allowed to bind to the immobilized antibody after incubation of the antibody with the sample. The extent to which components of the sample inhibit the binding of the labeled polypeptide to the antibody is indicative of the reactivity of the sample with the immobilized antibody, and as a result, indicative of the concentration of polypeptide in the sample.

The solid support may be any material known to those of ordinary skill in the art to which the antibody may be attached. For example, the solid support may be a test well in a microtiter plate or a nitrocellulose filter or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681.

The artibody may be immobilized on the solid support using a variety of techniques known to those in the art, which are amply described in the patent and scientific literature. In the context of the present invention, the term "immobilization" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the antigen and functional groups on the support or may be a linkage by way of a cross-linking agent). Immobilization by adsorption to a

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well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the antibody, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and 1 day. In general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of antibody ranging from about 10 ng to about 1 µg, and preferably about 100-200 ng, is sufficient to immobilize an adequate amount of polypeptide.

Covalent attachment of antibody to a solid support may also generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the antibody. For example, the antibody may be covalently attached to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the binding partner (see, e.g., Pierce Immunotechnology Catalog and Handbook (1991) at A12-A13).

In certain embodiments, the assay for detection of polypeptide in a sample is a two-antibody sandwich assay. This assay may be performed by first contacting an antibody that has been immobilized on a solid support, commonly the well of a microtiter plate, with the biological sample, such that the polypeptide within the sample are allowed to bind to the immobilized antibody. Unbound sample is then removed from the immobilized polypeptide antibody complexes and a second antibody (containing a reporter group) capable of binding to a different site on the polypeptide is added. The amount of second antibody that remains bound to the solid support is then determined using a method appropriate for the specific reporter group.

More specifically, once the antibody is immobilized on the support as described above, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin or Tween 20TM (Sigma Chemical Co., St. Louis, MO). The immobilized antibody is then incubated with the sample, and polypeptide is allowed to bind to the antibody. The sample may be diluted with a suitable diluent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact time (i.e., incubation

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time) is that period of time that is sufficient to detect the presence of polypeptide within a sample obtained from an individual with breast cancer. Preferably, the contact time is sufficient to achieve a level of binding that is at least 95% of that achieved at equilibrium between bound and unbound polypeptide. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20TM. The second antibody, which contains a reporter group, may then be added to the solid support. Preferred reporter groups include enzymes (such as horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. The conjugation of antibody to reporter group may be achieved using standard methods known to those of ordinary skill in the art.

The second antibody is then incubated with the immobilized antibodypolypeptide complex for an amount of time sufficient to detect the bound polypeptide.

An appropriate amount of time may generally be determined by assaying the level of
binding that occurs over a period of time. Unbound second antibody is then removed
and bound second antibody is detected using the reporter group. The method employed
for detecting the reporter group depends upon the nature of the reporter group. For
radioactive groups, scintillation counting or autoradiographic methods are generally
appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and
fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter
group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter
groups may generally be detected by the addition of substrate (generally for a specific
period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of breast cancer, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value established from non-tumor tissue. In one preferred embodiment, the cut-off value is the average mean signal

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obtained when the immobilized antibody is incubated with samples from patients without breast cancer. In general, a sample generating a signal that is three standard deviations above the predetermined cut-off value may be considered positive for breast cancer. In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett et al., Clinical Epidemiology: A Basic Science for Clinical Medicine, p. 106-7 (Little Brown and Co., 1985). Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (i.e., sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (i.e., the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for breast cancer.

In a related embodiment, the assay is performed in a flow-through or strip test format, wherein the antibody is immobilized on a membrane, such as nitrocellulose. In the flow-through test, the polypeptide within the sample bind to the immobilized antibody as the sample passes through the membrane. A second, labeled antibody then binds to the antibody-polypeptide complex as a solution containing the second antibody flows through the membrane. The detection of bound second antibody may then be performed as described above. In the strip test format, one end of the membrane to which antibody is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing second antibody and to the area of immobilized antibody. Concentration of second antibody at the area of immobilized antibody indicates the presence of breast cancer. Typically, the concentration of second antibody at that site generates a pattern, such as a line, that can be read visually. The absence of such a pattern indicates a negative result. In general, the amount of antibody immobilized on the membrane is selected to generate a visually

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discernible pattern when the biological sample contains a level of polypeptide that would be sufficient to generate a positive signal in the two-antibody sandwich assay, in the format discussed above. Preferably, the amount of antibody immobilized on the membrane ranges from about 25 ng to about 1 µg, and more preferably from about 50 ng to about 1 µg. Such tests can typically be performed with a very small amount of biological sample.

The presence or absence of breast cancer in a patient may also be determined by evaluating the level of mRNA encoding a breast tumor-specific polypeptide as described herein within the biological sample (e.g., a biopsy, mastectomy and/or blood sample from a patient) relative to a predetermined cut-off value. Such an evaluation may be achieved using any of a variety of methods known to those of ordinary skill in the art such as, for example, in situ hybridization and amplification by polymerase chain reaction.

For example, polymerase chain reaction may be used to amplify sequences from cDNA prepared from RNA that is isolated from one of the above biological samples. Sequence-specific primers for use in such amplification may be designed based on the sequences provided in any one cf SEQ ID NO: 1, 11-86, 142-298 301-303, 307, 313, 314, 316 and 317, and may be purchased or synthesized. In the case of B18Ag1, as noted herein, one suitable primer pair is B18Ag1-2 (5'ATG GCT ATT TTC GGG GGC TGA CA) (SEQ ID NO:126) and B18Ag1-3 (5'CCG GTA TCT CCT CGT GGG TAT T) (SEO ID NO:127). The PCR reaction products may then be separated by gel electrophoresis and visualized according to methods well known to those of ordinary skill in the art. Amplification is typically performed on samples obtained from matches pairs of tissue (tumor and non-tumor tissue from the same individual) or from unmatched pairs of tissue (tumor and non-tumor tissue from different individuals). The amplification reaction is preferably performed on several dilutions of cDNA spanning two orders of magnitude. A two-fold or greater increase in expression in several dilutions of the tumor sample as compared to the same dilution of the nontumor sample is considered positive.

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As used herein, the term "primer/probe specific for a polynucleotide" means an oligonucleotide sequence that has at least about 80% identity, preferably at least about 90% and more preferably at least about 95%, identity to the polynucleotide in question, or an eligonucleotide sequence that is anti-sense to a sequence that has at least about 80% identity, preferably at least about 90% and more preferably at least about 95%, identity to the polynucleotide in question. Primers and/or probes which may be usefully employed in the inventive diagnostic methods preferably have at least about 10-40 nucleotides. In a preferred embodiment, the polymerase chain reaction primers comprise at least about 10 contiguous nucleotides of a polynucleotide that encodes one of 10 the polypeptides disclosed herein or that is anti-sense to a sequence that encodes one of the polypeptides disclosed herein. Preferably, oligonucleotide probes for use in the inventive diagnostic methods comprise at least about 15 contiguous oligonucleotides of a polynucleotide that encodes one of the polyneptides disclosed herein or that is anti-sense to a sequence that encodes one of the polypeptides disclosed herein. Techniques for both PCR based assays and in situ hybridization assays are well known in the art.

Conventional RT-PCR protocols using agarose and ethidium bromide staining, while important in defining gene specificity, do not lend themselves to diagnostic kit development because of the time and effort required in making them quantitative (i.e., construction of saturation and/or titration curves), and their sample throughput. This problem is overcome by the development of procedures such as real time RT-PCR which allows for assays to be performed in single tubes, and in turn can be modified for use in 96 well plate formats. Instrumentation to perform such methodologies are available from Perkin Elmer/Applied Biosystems Division. Alternatively, other high throughput assays using labeled probes (e.g., digoxygenin) in combination with labeled (e.g., enzyme fluorescent, radioactive) antibodies to such probes can also be used in the development of 96 well plate assays.

In yet another method for determining the presence or absence of breast cancer in a patient, one or more of the breast tumor-specific polypeptides described may be used in a skin test. As used herein, a "skin test" is any assay performed directly on a patient in which a delayed-type hypersensitivity (DTH) reaction (such as swelling,

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reddening or dermatitis) is measured following intradermal injection of one or more polypeptides as described above. Such injection may be achieved using any suitable device sufficient to contact the polypeptide or polypeptides with dermal cells of the patient, such as a tuberculin syringe or 1 mL syringe. Preferably, the reaction is measured at least 48 hours after injection, more preferably 48-72 hours.

The DTH reaction is a cell-mediated immune response, which is greater in patients that have been exposed previously to a test antigen (i.e., an immunogenic portion of a polypeptide employed, or a variant thereof). The response may measured visually, using a ruler. In general, a response that is greater than about 0.5 cm in diameter, preferably greater than about 5.0 cm in diameter, is a positive response, indicative of ्रकीत और पि breast cancer.

The breast tumor-specific polypeptides described herein are preferably formulated, for use in a skin test, as pharmaceutical compositions containing at least one polypeptide and a physiologically acceptable carrier, such as water, saline, alcohol, or a buffer. Such compositions typically contain one or more of the above polypeptides in an amount ranging from about I µg to 100 µg, preferably from about 10 µg to 50 µg in a volume of 0.1 mL. Preferably, the carrier employed in such pharmaceutical compositions is a saline solution with appropriate preservatives, such as phenol and/or

In other aspects of the present invention, the progression and/or response to treatment of a breast cancer may be monitored by performing any of the above assays over a period of time, and evaluating the change in the level of the response (i.e., the amount of po ypeptide or mRNA detected or, in the case of a skin test, the extent of the immune response detected). For example, the assays may be performed every month to every other month for a period of 1 to 2 years. In general, breast cancer is progressing in those patients in whom the level of the response increases over time. In contrast, breast cancer is not progressing when the signal detected either remains constant or decreases with time.

In further aspects of the present invention, the compounds described herein may be used for the immunotherapy of breast cancer. In these aspects, the

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compounds (which may be polypeptides, antibodies or polynucleotides) are preferably incorporated into pharmaceutical compositions or vaccines. Pharmaceutical compositions comprise one or more such compounds and a physiologically acceptable carrier. Vaccines may comprise one or more such compounds in combination with an immunostimulant, such as an adjuvant or a liposome (into which the compound is incorporated). An immunostimulant may be any substance that enhances or potentiates an immune response (antibody and/or cell-mediated) to an exogenous antigen. Examples of immunostimulants include adjuvants, biodegradable microspheres (e.g., polylactic galactide) and liposomes (into which the compound is incorporated; see e.g., Fullerton, U.S. Patent No. 4,235,877). Vaccine preparation is generally described in, for example, M.F. Powell and M.J. Newman, eds., "Vaccine Design (the subunit and adjuvant approach)," Plenum Press (NY, 1995). Pharmaceutical compositions and vaccines within the scope of the present invention may also contain other compounds, which may be biologically active or inactive. For example, one or more immunogenic portions of other tumor antigens may be present, either incorporated into a fusion polypeptide or as a separate compound, within the composition or vaccine.

Alternatively, a vaccine may contain DNA encoding one or more of the polypeptides as described above, such that the polypeptide is generated in situ. In such vaccines, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as Bacillus-Calmette-Guerrin) that expresses an immunogenic portion of the polypeptide on its cell surface. In a preferred embodiment, the DNA may be introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective), replication competent virus. Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as described, for example, in Ulmer et al., Science 259:1745-1749 (1993), and reviewed by

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Cohen, Science 259:1691-1692 (1993). The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactate polyglycolate) may also be employed as carriers for the pharmaceutical compositions of this invention.

Any of a variety of immunostimulants may be employed in the vaccines of this invention. For example, an adjuvant may be included. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, Bortadella pertussis or Mycobacterium tuberculusis derived proteins. Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NI); AS-2 (SmithKline Beecham, Philadelphia, PA); aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryi lipid A and quil A. Cytokines, such as GM-CSF or interleukin-2, -7, or -12, may also be used as adjuvants.

Within the vaccines provided herein, the adjuvant composition is preferably designed to induce an immune response predominantly of the Th1 type. High levels of Th1-type cytokines (e.g., IFN-γ, TNFα, IL-2 and IL-12) tend to favor the induction of cell mediated immune responses to an administered antigen. In contrast, high levels of Th2-type cytokines (e.g., IL-4, IL-5, IL-6 and IL-10) tend to favor the

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induction of humoral immune responses. Following application of a vaccine as provided herein, a patient will support an immune response that includes Th1- and Th2-type responses. Within a preferred embodiment, in which a response is predominantly Th1type, the level of Th1-type cytokines will increase to a greater extent than the level of Th2-type cytokines. The levels of these cytokines may be readily assessed using standard assays. For a review of the families of cytokines, see Mosmann and Coffman, Ann. Rev. Immunol. 7:145-173, 1989.

Preferred adjuvants for use in eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-10 acylated monophosphoryl lipid A (3D-MPL), together with an aluminum salt. MPL adjuvants are available from Corixa Corporation (Seattle, WA; see US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555 and WO 99/33488. Immunostimulatory DNA sequences are also described, for example, by Sato et al., Science 273:352, 1996. Another preferred adjuvant is a saponin, preferably QS21 (Aquila Biopharmaceuticals Inc., Framingham, MA), which may be used alone or in combination with other adjuvants. For example, an enhanced system involves the combination of a monophosphoryl lipid A and saponin derivative, such as the combination of QS21 and 3D-MPL as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other preferred formulations comprise an oil-in-water emulsion and tocopherol. A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil-in-water emulsion is described in WO 95/17210, and a second secon

Other preferred adjuvants include Montanide ISA 720 (Seppic, France), SAF (Chiron, California, United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (e.g., SBAS-2 or SBAS-4, available from SmithKline Beecham, Rixensart, Belgium), Detox (Ribi ImmunoChem Research Inc., Hamilton, MT), RC-529 (Ribi ImmunoChem Research Inc., Hamilton, MT) and Aminoalkyl glucosaminide 4phosphates (AGPs).

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Any vaccine provided herein may be prepared using well known methods that result in a combination of antigen, immunostimulant and a suitable carrier or excipient. The compositions described herein may be administered as part of a sustained release formulation (i.e., a formulation such as a capsule, sponge or gel (composed of polysaccharides, for example) that effects a slow release of compound following administration). Such formulations may generally be prepared using well known technology (see, e.g., Coombes et al., Vaccine 14:1429-1438, 1996) and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a polypeptide, polynucleotide or antibody dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane.

Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. Such carriers include microparticles of poly(lactide-co-glycolide), as 15 well as polyacrylate, latex, starch, cellulose and dextran. Other delayed-release carriers include supramolecular biovectors, which comprise a non-liquid hydrophilic core (e.g., a cross-linked polysaccharide or oligosaccharide) and, optionally, an external layer comprising an amphiphilic compound, such as a phospholipid (see e.g., U.S. Patent No. 5,151,254 and PCT applications WO 94/20078, WO/94/23701 and WO 96/06638). The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Any of a variety of delivery vehicles may be employed within pharmaceutical compositions and vaccines to facilitate production of an antigen-specific immune response that targets tumor cells. Delivery vehicles include antigen presenting cells (APCs), such as dendritic cells, macrophages, B cells, monocytes and other cells that may be engineered to be efficient APCs. Such cells may, but need not, be genetically modified to increase the capacity for presenting the antigen, to improve activation and/or maintenance of the T cell response, to have anti-tumor effects per se and/or to be immunologically compatible with the receiver (i.e., matched HLA

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haplotype). APCs may generally be isolated from any of a variety of biological fluids and organs, including tumor and peritumoral tissues, and may be autologous, allogeneic, syngeneic or xenogeneic cells.

Certain preferred embodiments of the present invention use dendritic cells or progenitors thereof as antigen-presenting cells. Dendritic cells are highly potent APCs (Banchereau and Steinman, Nature 392:245-251, 1998) and have been shown to be effective as a physiological adjuvant for eliciting prophylactic or therapeutic antitumor immunity (see Timmerman and Levy, Ann. Rev. Med. 50:507-529, 1999). In general, dendritic cells may be identified based on their typical shape (stellate in situ, with marked cytoplasmic processes (dendrites) visible in vitro), their ability to take up, process and present antigens with high efficiency and their ability to activate naïve T cell responses. Dendritic cells may, of course, be engineered to express specific cell-surface receptors or ligands that are not commonly found on dendritic cells in vivo or ex vivo, and such modified dendritic cells are contemplated by the present invention. As an alternative to dendritic cells, secreted vesicles antigen-loaded dendritic cells (called exosomes) may be used within a vaccine (see Zitvogel et al., Nature Med. 4:594-600, 1998).

Dendritic cells and progenitors may be obtained from peripheral blood, bone marrow, numor-infiltrating cells, peritumoral tissues-infiltrating cells, lymph nodes, spleen, skin, umbilical cord blood or any other suitable tissue or fluid. For example, dendritic cells may be differentiated ex vivo by adding a combination of cytokines such as GM-CSF, II.-4, IL-13 and/or TNFα to cultures of monocytes harvested from peripheral blood. Alternatively, CD34 positive cells harvested from peripheral blood, umbilical cord blood or bone marrow may be differentiated into dendritic cells by adding to the culture medium combinations of GM-CSF, IL-3, TNFα, CD40 ligand, LPS, flt3 ligand and/or other compound(s) that induce differentiation, maturation and proliferation of dendritic cells.

Dendritic cells are conveniently categorized as "immature" and "mature" cells, which allows a simple way to discriminate between two well characterized phenotypes. However, this nomenclature should not be construed to exclude all possible

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intermediate stages of differentiation. Immature dendritic cells are characterized as APC with a high capacity for antigen uptake and processing, which correlates with the high expression of Fcy receptor and mannose receptor. The mature phenotype is typically characterized by a lower expression of these markers, but a high expression of cell surface molecules responsible for T cell activation such as class I and class II MHC, adhesion molecules (e.g., CD54 and CD11) and costimulatory molecules (e.g., CD40, CD80, CD86 and 4-1BB).

APCs may generally be transfected with a polynucleotide encoding a polypeptide of the present invention (or portion or other variant thereof) such that the polypeptide, or an immunogenic portion thereof, is expressed on the cell surface. Such transfection may take place ex vivo, and a composition or vaccine comprising such transfected cells may then be used for therapeutic purposes, as described herein. Alternatively, a gene delivery vehicle that targets a dendritic or other antigen presenting cell may be administered to a patient, resulting in transfection that occurs in vivo. In vivo and ex vivo transfection of dendritic cells, for example, may generally be performed using any methods known in the art, such as those described in WO 97/24447, or the gene gun approach described by Mahvi et al., Immunology and cell Biology 75:456-460, 1997. Antigen loading of generatic cells may be achieved by incubating dendritic cells or progenitor cells with the polypeptide, DNA (naked or within a plasmid vector) or RNA; or with antigen-expressing recombinant bacterium or viruses (e.g., vaccinia, fowlpox, adenovirus or lentivirus vectors). Prior to loading, the polypeptide may be covalently conjugated to an immunological partner that provides T cell help (e.g., a carrier molecule). Alternatively, a deadritic cell may be pulsed with a non-conjugated immunological parmer, separately or in the presence of the polypeptide.

Vaccines and pharmaceutical compositions may be presented in unit-dose or multi-dose containers, such as sealed ampoules or vials. Such containers are preferably hermetically sealed to preserve sterility of the formulation until use. In general, formulations may be stored as suspensions, solutions or emulsions in oily or aqueous vehicles. Alternatively, a vaccine or pharmaceutical composition may be stored in a freeze-dried condition requiring only the addition of a sterile liquid carrier

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immediately prior to use.

The above pharmaceutical compositions and vaccines may be used, for example, for the therapy of creast cancer in a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may or may not be afflicted with breast cancer. Accordingly, the above pharmaceutical compositions and vaccines may be used to prevent the development of breast cancer or to treat a patient afflicted with breast cancer. In a preferred embodiment, the compounds are administered either prior to or following surgical removal of primary tumors and/or treatment by administration of radiotherapy and conventional chemotherapeutic drugs. To prevent or slow the development of breast cancer, a pharmaceutical composition or vaccine comprising one or more polypeptides as described herein may be administered to a patient. Alternatively, naked DNA or plasmid or viral vector encoding the polypeptide may be administered. For treating a patient with breast cancer, the pharmaceutical composition or vaccine may comprise one or more polypeptides, antibodies or polynucleotides complementary to DNA encoding a polypeptide as described herein (e.g., antisense RNA or antisense deoxyribonucleotide oligonucleotides).

Routes and frequency of administration, as well as dosage, will vary from individual to individual. In general, the pharmaceutical compositions and vaccines may be administered by injection (e.g., intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (e.g., by aspiration) or orally. Between 1 and 10 doses may be administered for a 52-week period. Preferably, 6 doses are administered, at intervals of 1 month, and booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of a compound that, when administered as described above, is capable of promoting an antitumor immune response. Such response can be monitored by measuring the anti-tumor antibodies in a patient or by vaccine-dependent generation of cytolytic effector cells capable of killing the patient's tumor cells in vitro. Such vaccines should also be capable of causing an immune response that leads to an improved clinical outcome (e.g., more frequent remissions, complete or partial or longer disease-free survival) in vaccinated patients as compared to non-vaccinated patients. In general, for pharmaceutical

compositions and vaccines comprising one or more polypeptides, the amount of each polypeptide present in a dose ranges from about 100 µg to 5 mg. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL

Polypeptides disclosed herein may also be employed in adoptive immunotherapy for the treatment of cancer. Adoptive immunotherapy may be broadly classified into either active or passive immunotherapy. In active immunotherapy, treatment relies on the *in vivo* stimulation of the endogenous host immune system to react against tumors with the administration of immune response-modifying agents (for example, tumor vaccines, bacterial adjuvants, and/or cytokines).

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In passive immunotherapy, treatment involves the delivery of biologic reagents with established tumor-immune reactivity (such as effector cells or antibodies) that can directly or indirectly mediate antitumor effects and does not necessarily depend on an intact host immune system. Examples of effector cells include T lymphocytes (for example, CD8+ cytotoxic T-lymphocyte, CD4+ T-helper, tumor-infiltrating lymphocytes), killer cells (Natural Killer cells, lymphokine-activated killer cells), B cells, or antigen presenting cells (such as dendritic cells and macrophages) expressing the disclosed antigens. The polypeptides disclosed herein may also be used to generate antibodies or anti-idiotypic antibodies (as in U.S. Patent No. 4,918,164), for passive immunotherapy.

The predominant method of procuring adequate numbers of T-cells for adoptive immunotherapy is to grow immune T-cells in vitro. Culture conditions for expanding single antigen-specific T-cells to several billion in number with retention of antigen recognition in vivo are well known in the art. These in vitro culture conditions typically utilize intermittent stimulation with antigen, often in the presence of cytokines, such as IL-2, and non-dividing feeder cells. As noted above, the immunoreactive polypeptides described herein may be used to rapidly expand antigen-specific T cell cultures in order to generate sufficient number of cells for immunotherapy. In particular, antigen-presenting cells, such as dendritic, macrophage or B-cells, may be pulsed with immunoreactive polypeptides or transfected with a polynucleotide sequence(s), using

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standard techniques well known in the art. For cultured T-cells to be effective in therapy, the cultured T-cells must be able to grow and distribute widely and to survive long term in vivo. Studies have demonstrated that cultured T-cells can be induced to grow in vivo and to survive long term in substantial numbers by repeated stimulation with antigen supplemented with IL-2 (see, for example, Cheever et al. *Ibid*).

The polypeptides disclosed herein may also be employed to generate and/or isolate tumor-reactive T-cells, which can then be administered to the patient. In one technique, antigen-specific T-cell lines may be generated by *in vivo* immunization with short peptides corresponding to immunogenic portions of the disclosed polypeptides. The resulting antigen specific CD8+ CTL clones may be isolated from the patient, expanded using standard tissue culture techniques, and returned to the patient.

Alternatively, peptides corresponding to immunogenic portions of the polypeptides may be employed to generate tumor reactive T cell subsets by selective *in vitro* stimulation and expansion of autologous T cells to provide antigen-specific T cells which may be subsequently transferred to the patient as described, for example, by Chang et al. (Crit. Rev. Oncol. Hematol., 22(3), 213, 1996).

In another embodiment, syngeneic or autologous dendritic cells may be pulsed with peptides corresponding to at least an immunogenic portion of a polypeptide disclosed herein. The resulting antigen-specific dendritic cells may either be transferred into a patient, or employed to stimulate T cells to provide antigen-specific T cells which may, in turn, be administered to a patient. The use of peptide-pulsed dendritic cells to generate antigen-specific T cells and the subsequent use of such antigen-specific T cells to eradicate tumors in a murine model has been demonstrated by Cheever et al. ("Therapy With Cultured T Cells: Principles Revisited," *Immunological Reviews*, 157:177, 1997).

Additionally vectors expressing the disclosed polynucleotides may be introduced into stem cells taken from the patient and clonally propagated *in vitro* for autologous transplant back into the same patient. In one embodiment, cells of the immune system, such as T cells, may be isolated from the peripheral blood of a patient, using a commercially available cell separation system, such as CellPro Incorporated's (Bothell,

WA) CEPRATE™ system (see U.S. Patent No. 5,240,856; U.S. Patent No. 5,215,926; WO 89/06280; WO 91/16116 and WO 92/07243). The separated cells are stimulated with one or more of the immunoreactive polypeptides contained within a delivery vehicle, such as a microsphere, to provide antigen-specific T cells. The population of tumor antigen-specific T cells is then expanded using standard techniques and the cells are administered back to the patient.

The following Examples are offered by way of illustration and not by way of limitation.

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EXAMPLES

EXAMPLE 1

PREPARATION OF BREAS I TUMOR-SPECIFIC CDNAS USING DIFFERENTIAL DISPLAY RT-PCR

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This Example illustrates the preparation of cDNA molecules encoding breast tumor-specific polypeptides using a differential display screen.

A. Preparation of B18Ag1 cDNA and Characterization of mRNA Expression

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Tissue samples were prepared from breast tumor and normal tissue of a patient with breast cancer that was confirmed by pathology after removal from the patient. Normal RNA and tumor RNA was extracted from the samples and mRNA was isolated and converted into cDNA using a (dT)₁₂AG (SEQ ID NO:130) anchored 3' primer. Differential display PCR was then executed using a randomly chosen primer (CTTCAACCTC) (SEQ ID NO:103). Amplification conditions were standard buffer containing 1.5 mM MgCl₂, 20 pmol of primer, 500 pmol dNTP, and 1 unit of *Taq* DNA polymerase (Perkin-Elmer, Branchburg, NJ). Forty cycles of amplification were performed using 94°C denaturation for 30 seconds, 42°C annealing for 1 minute, and 72° C extension for 30 seconds. An RNA fingerprint containing 76 amplified products was

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obtained. Although the RNA fingerprint of breast tumor tissue was over 98% identical to that of the normal breast tissue, a band was repeatedly observed to be specific to the RNA fingerprint pattern of the tumor. This band was cut out of a silver stained gel, subcloned into the T-vector (Novagen, Madison, WI) and sequenced.

The sequence of the cDNA, referred to as B18Ag1, is provided in SEQ ID NO:1. A database search of GENBANK and EMBL revealed that the B18Ag1 fragment initially cloned is 77% identical to the endogenous human retroviral element S71, which is a truncated retroviral element homologous to the Simian Sarcoma Virus (SSV). S71 contains an incomplete gag gene, a portion of the pol gene and an LTR-like structure at the 3' terminus (see Werner et al., Virology 174:225-238 (1990)). B18Ag1 is also 64% identical to SSV in the region corresponding to the P30 (gag) locus. B18Ag1 contains three separate and incomplete reading frames covering a region which shares considerable homology to a wide variety of gag proteins of retroviruses which infect mammals. In addition, the homology to S71 is not just within the gag gene, but spans several kb of sequence including an LTR.

B18Ag1-specific PCR primers were synthesized using computer analysis guidelines. RT-PCR amplification (94°C, 30 seconds; 60°C \rightarrow 42°C, 30 seconds; 72°C, 30 seconds for 40 cycles) confirmed that B18Ag1 represents an actual mRNA sequence present at relatively high levels in the patient's breast tumor tissue. The primers used in amplification were B18Ag1-1 (CTG CCT GAG CCA CAA ATG) (SEQ ID NO:128) and B18Ag1-4 (CCG GAG GAG GAA GCT AGA GGA ATA) (SEQ ID NO:129) at a 3.5 mM magnesium concentration and a pH of 8.5, and B18Ag1-2 (ATG GCT ATT TTC GGG GCC TGA CA) (SEQ ID NO:126) and B18Ag1-3 (CCG GTA TCT CCT CGT GGG TAT T) (SEQ ID NO:127) at 2 mM magnesium at pH 9.5. The same experiments showed exceedingly low to nonexistent levels of expression in this patient's normal breast tissue (see Figure 1). RT-PCR experiments were then used to show that B18Ag1 mRNA is present in nine other breast tumor samples (from Brazilian and American patients) but absent in, or at exceedingly low levels in, the normal breast tissue corresponding to each cancer patient. RT-PCR analysis has also shown that the B18Ag1 transcript is not present in various normal tissues (including lymph node, myocardium

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and liver) and present at relatively low levels in PBMC and lung tissue. The presence of B18Ag1 mRNA in breast tumor samples, and its absence from normal breast tissue, has been confirmed by Northern blot analysis, as shown in Figure 2.

The differential expression of B18Ag1 in breast tumor tissue was also confirmed by RNase protection assays. Figure 3 shows the level of B18Ag1 mRNA in various tissue types as determined in four different RNase protection assays. Lanes 1-12 represent various normal breast tissue samples, lanes 13-25 represent various breast tumor samples; lanes 26-27 represent normal prostate samples; lanes 28-29 represent prostate tumor samples; lanes 30-32 represent colon tumor samples; lane 33 represents normal aorta; lane 34 represents normal small intestine; lane 35 represents normal skin, lane 36 represents normal lymph node; lane 37 represents normal ovary; lane 38 represents normal liver; lane 39 represents normal skeletal muscle; lane 40 represents a first normal stomach sample, lane 41 represents a second normal stomach sample; lane 42 represents a normal lung; lane 43 represents normal kidney; and lane 44 represents normal pancreas. Interexperimental comparison was facilitated by including a positive control RNA of known β-actin message abundance in each assay and normalizing the results of the different assays with respect to this positive control.

RT-PCR and Southern Blot analysis has shown the B18Ag1 locus to be present in human genomic DNA as a single copy endogenous retroviral element. A genomic clone of approximately 12-18 kb was isolated using the initial B18Ag1 sequence as a probe. Four additional subclones were also isolated by XbaI digestion. Additional retroviral sequences obtained from the ends of the XbaI digests of these clones (located as shown in Figure 4) are shown as SEQ ID NO:3 - SEQ ID NO:10, where SEQ ID NO:3 shows the location of the sequence labeled 10 in Figure 4, SEQ ID NO:4 shows the location of the sequence labeled 11-29, SEQ ID NO:5 shows the location of the sequence labeled 6, SEQ ID NO:7 shows the location of the sequence labeled 12, SEQ ID NO:8 shows the location of the sequence labeled 13, SEQ ID NO:9 shows the location of the sequence labeled 11-

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Subsequent studies demonstrated that the 12-18 kb genomic clone contains a retroviral element of about 7.75 kb, as shown in Figures 5A and 5B. The sequence of this retroviral element is shown in SEQ ID NO: 141. The numbered line at the top of Figure 5A represents the sense strand sequence of the retroviral genomic clone. The box below this line shows the position of selected restriction sites. The arrows depict the different overlapping clones used to sequence the retroviral element. The direction of the arrow shows whether the single-pass subclone sequence corresponded to the sense or anti-sense strand. Figure 5B is a schematic diagram of the retroviral element containing B18Ag1 depicting the organization of viral genes within the element. The open boxes correspond to predicted reading frames, starting with a methionine, found throughout the element. Each of the six likely reading frames is shown, as indicated to the left of the boxes, with frames 1-3 corresponding to those found on the sense strand.

Using the cDNA of SEQ ID NO:1 as a probe, a longer cDNA was obtained (SEQ ID NO:227) which contains minor nucleotide differences (less than 1%) compared to the genomic sequence shown in SEQ ID NO:141.

B. <u>Preparation of cDNA Molecules Encoding Other Breast Tumor-Specific</u> Polypeptides

Normal RNA and tumor RNA was prepared and mRNA was isolated and converted into cDNA using a (dT)₁₂AG anchored 3' primer, as described above. Differential display PCR was then executed using the randomly chosen primers of SEQ ID NO: 87-125. Amplification conditions were as noted above, and bands observed to be specific to the RNA fingerprint pattern of the tumor were cut out of a silver stained gel, subcloned into either the T-vector (Novagen, Madison, WI) or the pCRII vector (Invitrogen, San Diego, CA) and sequenced. The sequences are provided in SEQ ID NO:11 - SEQ ID NO:86. Of the 79 sequences isolated, 67 were found to be novel (SEQ ID NO:11-26 and 28-77) (see also Figures 6-20).

An extended DNA sequence (SEQ ID NO: 290) for the antigen B15Ag1 (originally identified partial sequence provided in SEQ ID NO: 27) was obtained in further studies. Comparison of the sequence of SEQ ID NO: 290 with those in the gene bank as described above, revealed homology to the known human β-A activin gene.

Further studies led to the isolation of the full-length cDNA sequence for the antigen B21GT2 (also referred to as B311D; originally identified partial cDNA sequence provided in SEQ ID NO: 56). The full-length sequence is provided in SEQ ID NO: 307, with the corresponding amino acid sequence being provided in SEQ ID NO: 308. Further studies led to the isolation of a splice variant of B311D. The B311D clone of SEQ ID NO: 316 was sequenced and a Xhol/NotI fragment from this clone was gel purified and 32P-cDTP labeled by random priming for use as a probe for further screening to obtain additional B311D gene sequence. Two fractions of a human breast tumor cDNA bacterial library were screened using standard techniques. One of the clones isolated in this manner yielded additional sequence which includes a poly A+ tail. The determined cDNA sequence of this clone (referred to as B311D_BT1_1A) is provided in SEQ ID NO: 317. The sequences of SEQ ID NO: 316 and 317 were found to share identity over a 464 bp region, with the sequences diverging near the poly A+ sequence of SEQ ID NO: 317.

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Subsequent studies identified an additional 146 sequences (SEQ ID NOS:142-289), of which 115 appeared to be novel (SEQ ID NOS:142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288 and 291). To the best of the inventors' knowledge none of the previously identified sequences have heretofore been shown to be expressed at a greater level in human breast tumor tissue than in normal breast tissue.

In further studies, several different splice forms of the antigen B11Ag1 (also referred to as B305D) were isolated, with each of the various splice forms containing slightly different versions of the B11Ag1 coding frame. Splice junction sequences define individual exons which, in various patterns and arrangements, make up the various splice forms. Primers were designed to examine the expression pattern of each of the exons using RT-PCR as described below. Each exon was found to show the same expression pattern as the original B11Ag1 clone, with expression being breast tumor-, normal prostate- and normal testis-specific. The determined cDNA sequences for the isolated protein coding exons are provided in SEQ ID NO: 292-298, respectively.

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The predicted amino acid sequences corresponding to the sequences of SEQ ID NO: 292 and 298 are provided in SEQ ID NO: 299 and 300. Additional studies using rapid amplification of cDNA ends (RACE), a 5' specific primer to one of the splice forms of B11Ag1 provided above and a breast adenocarcinoma, led to the isolation of three additional, related, splice forms referred to as isoforms B11C-15, B11C-8 and B11C-9,16. The determined cDNA sequences for these isoforms are provided in SEQ ID NO: 301-303, with the corresponding predicted amino acid sequences being provided in SEQ ID NO: 304-306.

In subsequent studies on B305D isoform A (cDNA sequence provided in SEQ ID NO: 292), the cDNA sequence (provided in SEQ ID NO: 313) was found to contain an additional guarante residue at position 884, leading to a frameshift in the open reading frame. The determined DNA sequence of this ORF is provided in SEQ ID NO: 314. This frameshift generates a protein sequence (provided in SEQ ID NO: 315) of 293 amino acids that contains the C-terminal domain common to the other isoforms of B305D but that differs in the N-terminal region.

EXAMPLE 2

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PREPARATION OF B18AG1 DNA FROM HUMAN GENOMIC DNA

This Example illustrates the preparation of B18Ag1 DNA by amplification from human genomic DNA.

B18Ag1 DNA may be prepared from 250 ng human genomic DNA using 20 pmol of B18Ag1 specific primers, 500 pmol dNTPS and 1 unit of *Taq* DNA polymerase (Perkin Elmer, Branchburg, NJ) using the following amplification parameters: 94°C for 30 seconds denaturing, 30 seconds 60°C to 42°C touchdown annealing in 2°C increments every two cycles and 72°C extension for 30 seconds. The last increment (a 42°C annealing temperature) should cycle 25 times. Primers were selected using computer analysis. Primers synthesized were B18Ag1-1, B18Ag1-2, B18Ag1-3, and B18Ag1-4. Primer pairs that may be used are 1+3, 1+4, 2+3, and 2+4.

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Following gel electrophoresis, the band corresponding to B18Ag1 DNA may be excised and cloned into a suitable vector.

EXAMPLE 3

PREPARATION OF B18AG1 DNA FROM BREAST TUMOR CDNA

This Example illustrates the preparation of B18Ag1 DNA by amplification from human breast tumor cDNA.

First strand cDNA is synthesized from RNA prepared from human breast tumor tissue in a reaction mixture containing 500 ng poly A+ RNA, 200 pmol of the primer (T)₁₂AG (i.e., TTT TTT TTT TTT AG) (SEQ ID NO: 130), 1X first strand reverse transcriptase buffer, 6.7 mM DTT, 500 mmol dNTPs, and 1 unit AMV or MMLV reverse transcriptase (from any supplier, such as Gibco-BRL (Grand Island, NY)) in a final volume of 30 μl. After first strand synthesis, the cDNA is diluted approximately 25 fold and 1 μl is used for amplification as described in Example 2. While some primer pairs can result in a heterogeneous population of transcripts, the primers B18Ag1-2 (5'ATG GCT ATT TTC GGG GGC TGA CA) (SEQ ID NO: 126) and B18Ag1-3 (5'CCG GTA TCT CCT CGT GGG TAT T) (SEQ ID NO: 127) yield a single 151 bp amplification product.

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EXAMPLE 4

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IDENTIFICATION OF B-CELL AND T-CELL EPITOPES OF B18AG1

This Example illustrates the identification of B18Ag1 epitopes.

The B18Ag1 sequence can be screened using a variety of computer algorithms. To determine B-cell epitopes, the sequence can be screened for hydrophobicity and hydrophilicity values using the method of Hopp, *Prog. Clin. Biol. Res. 172B*:367-77 (1985) or, alternatively, Cease et al., *J. Exp. Med. 164*:1779-84 (1986) or Spouge et al., *J. Immunol. 138*:204-12 (1987). Additional Class II MHC (antibody or B-cell) epitopes can be predicted using programs such as AMPHI (e.g., Margalit et al., *J.*

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Immunol. 138:2213 (1987)) or the methods of Rothbard and Taylor (e.g., EMBO J. 7:93 (1988)).

Once peptides (15-20 amino acids long) are identified using these techniques, individual peptides can be synthesized using automated peptide synthesis equipment (available from manufacturers such as Perkin Elmer/Applied Biosystems Division, Foster City, CA) and techniques such as Merrifield synthesis. Following synthesis, the peptides can used to screen sera harvested from either normal or breast cancer patients to determine whether patients with breast cancer possess antibodies reactive with the peptides. Presence of such antibodies in breast cancer patient would confirm the immunogenicity of the specific B-cell epitope in question. The peptides can also be tested for their ability to generate a serologic or humoral immune in animals (mice, rats, rabbits, chimps etc.) following immunization in vivo. Generation of a peptide-specific antiserum following such immunization further confirms the immunogenicity of the specific B-cell epitope in question.

To identify T-cell epitopes, the B18Ag1 sequence can be screened using different computer algorithms which are useful in identifying 8-10 amino acid motifs within the B18Ag1 sequence which are capable of binding to HLA Class I MHC molecules. (see, e.g., Rammensee et al., Immunogenetics 41:178-228 (1995)). Following synthesis such peptides can be tested for their ability to bind to class I MHC using standard binding assays (e.g., Sette et al., J. Immunol. 153:5586-92 (1994)) and more importantly can be tested for their ability to generate antigen reactive cytotoxic T-cells following in vitro stimulation of patient or normal peripheral mononuclear cells using, for example, the methods of Bakker et al., Cancer Res. 55:5330-34 (1995); Visseren et al., J. Immunol. 154:3991-98 (1995); Kawakami et al., J. Immunol. 154:3961-68 (1995); and Kast et al., J. Immunol. 152:3904-12 (1994). Successful in vitro generation of T-cells capable of killing autologous (bearing the same Class I MHC molecules) tumor cells following in vitro peptide stimulation further confirms the immunogenicity of the B18Ag1 antigen. Furthermore, such peptides may be used to generate murine peptide and B18Ag1 reactive cytotoxic T-cells following in vivo immunization in mice rendered

transgenic for expression of a particular human MHC Class I haplotype (Vitiello et al., J. Exp. Med. 173:1007-15 (1991).

A representative list of predicted B18Ag1 B-cell and T-cell epitopes, broken down according to predicted HLA Class I MHC binding antigen, is shown below:

Predicted Th Motifs (B-ceil epitopes) (SEQ ID NOS.: 131-133)

SSGGRTFDDFHRYLLVG

QGAAQKPINLSKXIEVVQGHDE

SPGVFLEHLQEAYRIYTPFDLSA

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Predicted HLA A2.1 Motifs (T-cell epitopes) (SEQ ID NOS.: 134-140)

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YLLVGIQGA

GAAQKPINL

NLSKXIEVV

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EVVQGHDES

HLQEAYRIY

NLAFVAOAA

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IDENTIFICATION OF T-CELL EPITOPES OF B11AG1

This Example illustrates the identification of B11Ag1 (also referred to as B305D) epitopes. Four peptides, referred to as B11-8, B11-1, B11-5 and B11-12 (SEQ ID NO: 309-312, respectfully) were derived from the B11Ag1 gene.

Human CD8 T cells were primed in vitro to the peptide B11-8 using dendritic cells according to the protocol of Van Tsai et al. (Critical Reviews in Immunology 18:65-75, 1998). The resulting CD8 T cell cultures were tested for their ability to recognize the B11-8 peptide or a negative control peptide, presented by the B-LCL line, JY. Briefly, T cells were incubated with autologous monocytes in the presence of 10 ug/ml peptide, 10 ng/ml IL-7 and 10 ug/ml IL-2, and assayed for their ability to

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specifically lyse target cells in a standard 51-Cr release assay. As shown in Fig. 22, the bulk culture line demonstrated strong recognition of the B11-8 peptide with weaker recognition of the peptide B11-1.

A clone from this CTL line was isolated following rapid expansion using the monoclonal antibody OKT3 and human IL-2. As shown in Fig. 23, this clone (referred to as A1), in addition to being able to recognize specific peptide, recognized JY LCL transduced with the B11Ag1 gene. This data demonstrates that B11-8 is a naturally processed epitope of the B11Ag1 gene. In addition these T cells were further found to recognize and lyse, in an HLA-A2 restricted manner, an established tumor cell line naturally expressing B11Ag1 (Fig. 24). The T cells strongly recognize a lung adenocarcinoma (LT-140-22) naturally expressing B11Ag1 transduced with HLA-A2, as well as an A2+ breast carcinoma (CAMA-1) transduced with B11Ag1, but not untransduced lines or another negative tumor line (SW620).

These data clearly demonstrate that these human T cells recognize not only B11-specific peptides but also transduced cells, as well as naturally expressing tumor lines.

CTL lines raised against the antigens B11-5 and B11-12, using the procedures described above, were found to recognize corresponding peptide-coated targets.

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Example 6

CHARACTERIZATION OF BREAST TUMOR GENES DISCOVERED BY DIFFERENTIAL DISPLAY PCR

The specificity and sensitivity of the breast tumor genes discovered by differential display PCR were determined using RT-PCR. This procedure enabled the rapid evaluation of breast tumor gene mRNA expression semiquantitatively without using large amounts of RNA. Using gene specific primers, mRNA expression levels in a variety of tissues were examined, including 8 breast tumors, 5 normal breasts, 2 prostate tumors, 2 colon tumors, 1 lung tumor, and 14 other normal adult human tissues, including normal prostate, colon, kidney, liver, lung, ovary, pancreas, skeletal muscle, skin, stomach and testes.

To ensure the semiquantitative nature of the RT-PCR, β -actin was used as internal control for each of the tissues examined. Serial dilutions of the first strand cDNAs were prepared and RT-PCR assays performed using β -actin specific primers. A dilution was then selected that enabled the linear range amplification of β -actin template, and which was sensitive enough to reflect the difference in the initial copy number. Using this condition, the β -actin levels were determined for each reverse transcription reaction from each tissue. DNA contamination was minimized by DNase treatment and by assuring a negative result when using first strand cDNA that was prepared without adding reverse transcriptase.

Using gene specific primers, the mRNA expression levels were determined in a variety of tissues. To date, 38 genes have been successfully examined by RT-PCR, five of which exhibit good specificity and sensitivity for breast tumors (B15AG-1, B31GA1b, B38GA2a, B11A1a and B18AG1a). Figures 21A and 21B depict the results for three of these genes: B15AG-1 (SEQ ID NO:27), B31GA1b (SEQ ID NO:148) and B38GA2a (SEQ ID NO. 157). Table I summarizes the expression level of all the genes tested in normal breast tissue and breast tumors, and also in other tissues.

TABLE I

Percentage of Breast Cancer Antigens that are Expressed in Various Tissues

Breast Tissues	Over-expressed in Breast Tumors	84%	
	Equally Expressed in Normals and Tumor	16%	
)	Over-expressed in Breast Tumors but not in any Normal Tissues	9%	
	Over-expressed in Breast Tumors but Expressed in Some Normal Tissues	30%	
	Over-expressed in Breast Tumors but Equally Expressed in All Other Tissues	61%	

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

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CLAIMS

- 1. An isolated polypeptide, comprising at least an immunogenic portion of a protein, or a variant thereof, wherein the protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:
 - (a) sequences recited in SEQ ID NOs: 1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317;
 - (b) sequences that hybridize to a sequence recited in any one of SEQ ID NOs:_ 1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317 under moderately stringent conditions; and
 - (c) complements of sequences of (a) or (b).
- An isolated polypeptide according to claim 1, wherein the polypeptide comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs: 1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317 or a complement of any of the foregoing polynucleotide sequences.
 - 3. An isolated polypeptide comprising a sequence recited in any one of SEQ ID NOs: 299, 300, 304-306, 308 and 315.
 - 4. An isolated polynucleotide encoding at least 15 amino acid

residues of a protein, or a variant thereof that differs in one or more substitutions, deletions, additions and/or insertions such that the ability of the variant to react with antigen-specific antisera is not substantially diminished, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide comprising a sequence recited in any one of SEQ ID NOs:_1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317 or a complement of any of the foregoing sequences.

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5. An isolated polynucleotide encoding a protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide comprising a sequence recited in any one of SEQ ID NOs: 1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317 or a complement of any of the foregoing sequences.

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composition from the

- 6. An isolated polynucleotide, comprising a sequence recited in any one of SEQ ID Nos: 1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317.
- 7. An isolated polynucleotide, comprising a sequence that hybridizes to a sequence recited in any one of SEQ ID NOs: 1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317 under moderately stringent conditions.



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- 8. An isolated polynucleotide complementary to a polynucleotide according to any one of claims 4-7.
- 9. An expression vector, comprising a polynucleotide according to any one of claims claim 4-8.
 - 10. A host cell transformed or transfected with an expression vector according to claim 9.
- 11. An isolated antibody, or antigen-binding fragment thereof, that specifically binds to a protein that comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs: 1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317 or a complement of any of the foregoing polynucleotide sequences.
 - 12. A fusion protein, comprising at least one polypeptide according to claim 1.

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- 13. A fusion protein according to claim 12, wherein the fusion protein comprises an expression enhancer that increases expression of the fusion protein in a host cell transfected with a polynucleotide encoding the fusion protein.
- 25 14. A fusion protein according to claim 12, wherein the fusion protein comprises a T helper epitope that is not present within the polypeptide of claim 1.
- 15. A fusion protein according to claim 12, wherein the fusion protein comprises an affinity tag.

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- 16. An isolated polynucleotide encoding a fusion protein according to claim 12.
- 17. A pharmaceutical composition, comprising a physiologically acceptable carrier and at least one component selected from the group consisting of:
 - (a) a polypeptide according to claim 1;
 - (b) a polynucleotide according to claim 4;
 - (c) an antibody according to claim 11;
 - (d) a fusion protein according to claim 12; and
- (e) a polynucleotide according to claim 16.
 - 18. A vaccine comprising an immunostimulant and at least one component selected from the group consisting of:
 - (a) a polypeptide according to claim 1;
 - (b) a polynucleotide according to claim 4;
 - (c) an antibody according to claim 11;
 - (d) a fusion protein according to claim 12; and
 - (e) a polynucleotide according to claim 16.
 - 19. A vaccine according to claim 18, wherein the immunostimulant is an adjuvant.
 - 20. A vaccine according to any claim 18, wherein the immunostimulant induces a predominantly Type I response.
 - 21. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a pharmaceutical composition according to claim 17.
- 22. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a vaccine according to

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claim 18.

- 23. A pharmaceutical composition comprising an antigen-presenting cell that expresses a polypeptide according to claim 1, in combination with a pharmaceutically acceptable carrier or excipient.
- 24. A pharmaceutical composition according to claim 23, wherein the antigen presenting cell is a dendritic cell or a macrophage.
- 25. A vaccine comprising an antigen-presenting cell that expresses a polypeptide comprising at least an immunogenic portion of a protein, or a variant thereof, wherein the protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:
 - (a) sequences recited in SEQ ID NOs: 1, 3-26, 28-86, 142-253, 255-298, 301-303, 307, 313, 314, 316 and 317;
 - (b) sequences that hybridize to a sequence recited in any one of SEQ ID NOs:_1, 3-26, 28-86, 142-253, 255-298, 301-303, 307, 313, 314, 316 and 317 under moderately stringent conditions; and
 - (c) complements of sequences of (i) or (ii); in combination with an immunostimulant.
 - 26. A vaccine according to claim 25, wherein the immunostimulant is an adjuvant.

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- 27. A vaccine according to claim 25, wherein the immunostimulant induces a predominantly Type I response.
 - 28. A vaccine according to claim 25, wherein the antigen-presenting cell is a dendritic cell.
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29. A method for inhibiting the development of a cancer in a patient,

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comprising administering to a patient an effective amount of an antigen-presenting cell that expresses a polypeptide comprising at least an immunogenic portion of a protein, or a variant thereof, wherein the protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

- (a) sequences recited in SEQ ID NOs: 1, 3-26, 28-86, 142-253, 255-298, 301-303, 307, 313, 314, 316 and 317;
- (b) sequences that hybridize to a sequence recited in any one of SEQ ID NOs: 1, 3-26, 28-86, 142-253, 255-298, 301-303, 307, 313, 314, 316 and 317 under moderately stringent conditions; and
- (c) complements of sequences encoded by a polynucleotide recited and any one of SEQ ID NOs: 1, 3-26, 28-86, 142-253, 255-298, 301-303, 307, 313, 314, 316 and 317;

and thereby inhibiting the development of a cancer in the patient.

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- 30. A method according to claim 29, wherein the antigen-presenting cell is a dendritic cell.
- the cancer is breast cancer.

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- comprising contacting a biological sample with T cells that specifically react with a protein, wherein the protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of
- (i) polynucleotides recited in any one of SEQ ID NOs: 1, 3-26, 28-86, 142-253, 255-298, 301-303, 307, 313, 314, 316 and 317; and
 - (ii) complements of the foregoing polynucleotides;

wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the antigen from the sample.

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33. A method according to claim 32, wherein the biological sample is

blood or a fraction thereof.

34. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient a biological sample treated according to the method of claim 32. A BOK OF OF A Secretary was a second

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35. A method for stimulating and/or expanding T cells specific for a protein, comprising contacting T cells with at least one component selected from the The should be an inches a common of group consisting of:

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- (a) polypeptides comprising at least an immunogenic portion of a protein, or a variant thereof, wherein the protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:
 - sequences recited in SEQ ID NOs: 1, 3-26, 28-86, 142-253, 255-298, 301-303, 307, 313, 314, 316 and 317;
- 15 sequences that hybridize to a sequence recited in any one of SEQ ID NOs: 1, 3-26, 28-86, 142-253, 255-298, 301-303, 307, 313, 314, 316 and 317 under moderately stringent conditions; and
- (iii) complements of sequences of (i) or (ii);
 - polynucleotides encoding a polypeptide of (a); and (b)
- antigen presenting cells that express a polypeptide of (a); (c) under conditions and for a time sufficient to permit the stimulation and/or expansion of T cells.
 - 36. An isolated T cell population, comprising T cells prepared according to the method of claim 35. 25

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37. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a T cell population according to claim 36.

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comprising the steps of:

- (a) incubating CD4⁺ and/or CD8+ T cells isolated from a patient with at least one component selected from the group consisting of:
 - (i) polypeptides comprising at least an immunogenic portion of a protein, or a variant thereof, wherein the protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:
 - (1) sequences recited in SEQ ID NOs: 1, 3-26, 28-86, 142-253, 255-298, 301-303, 307, 313, 314, 316 and 317;
 - (2) sequences that hybridize to a sequence recited in any one of SEQ ID NOs: 1, 3-26, 28-86, 142-253, 255-298, 301-303, 307, 313, 314, 316 and 317 under moderately stringent conditions; and
 - (3) complements of sequences of (1) or (2);
 - (ii) polynucleotides encoding a polypeptide of (i); and
- (iii) antigen presenting cells that expresses a polypeptide of (i);

such that T cells proliferate; and

- (b) administering to the patient an effective amount of the proliferated T cells, and thereby inhibiting the development of a cancer in the patient.
- 39. A method for inhibiting the development of a cancer in a patient, comprising the steps of:

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- (a) incubating CD4⁺ and/or CD8+, T cells isolated from a patient with at least one component selected from the group consisting of:
- (i) polypeptides comprising at least an immunogenic portion of a protein, or a variant thereof, wherein the protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:
 - (1) sequences recited in SEQ ID NOs: 1, 3-26, 28-86, 142-253, 255-298, 301-303, 307, 313, 314, 316 and 317;
 - (2) sequences that hybridize to a sequence recited in

any one of SEQ ID NOs:_ 1, 3-26, 28-86, 142-253, 255-298, 301-303, 307, 313, 314, 316 and 317 under moderately stringent conditions; and

- (3) complements of sequences of (1) or (2);
- (ii) polynucleotides encoding a polypeptide of (i); and
 - (iii) antigen presenting cells that express a polypeptide

of (i);

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such that T cells proliferate;

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- (b) cloning at least one proliferated cell to provide cloned T cells; and
- 10 (c) administering to the patient an effective amount of the cloned T cells, and thereby inhibiting the development of a cancer in the patient.
 - 40. A method for determining the presence or absence of a cancer in a patient, comprising the steps of:
 - (a) contacting a biological sample obtained from a patient with a binding agent that binds to a protein, wherein the protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs: 1, 3-26, 28-86, 142-253, 255-298, 301-303, 307, 313, 314, 316 and 317 or a complement of any of the foregoing polynucleotide sequences;
- 20 (b) detecting in the sample an amount of polypeptide that binds to the binding agent; and
 - (c) comparing the amount of polypeptide to a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient.
- 25 41. A method according to claim 40, wherein the binding agent is an antibody.
 - 42. A method according to claim 43, wherein the antibody is a monoclonal antibody.
 - 43. A method according to claim 40, wherein the cancer is breast

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cancer.

- 44. A method for monitoring the progression of a cancer in a patient, comprising the steps of:
- 5 (a) contacting a biological sample obtained from a patient at a first point in time with a binding agent that binds to a protein, wherein the protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs: 1, 3-26, 28-86, 142-253, 255-298, 301-303, 307, 313, 314, 316 and 317 or a complement of any of the foregoing polynucleotide sequences;
- 10° 10° 10° 10° 10° 10° detecting in the sample an amount of polypeptide that binds to the binding agent;
 - (c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and
- (d) comparing the amount of polypeptide detected in step (c) to the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

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45. A method according to claim 44, wherein the binding agent is an antibody.

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- 46. A method according to claim 45, wherein the antibody is a monoclonal antibody.
- 47. A method according to claim 44, wherein the cancer is a breast cancer.

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- 48. A method for determining the presence or absence of a cancer in a patient, comprising the steps of:
- (a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a protein, wherein the protein comprises an amino acid sequence that is encoded by a polynucleotide sequence

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recited in any one of SEQ ID NO: 1, 3-26, 28-86, 142-253, 255-298, 301-303, 307, 313, 314, 316 and 317 or a complement of any of the foregoing polynucleotide sequences;

- (b) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide; and
- (c) comparing the amount of polynucleotide that hybridizes to the oligonucleotide to a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient.

49. A method according to claim 48, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a polymerase chain reaction.

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- 50. A method according to claim 48, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a hybridization assay.
- 51. A method for monitoring the progression of a cancer in a patient, comprising the steps of:
- (a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a protein, wherein the protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NO: 1, 3-26, 28-86, 142-253, 255-298, 301-303, 307, 313, 314, 316 and 317 or a complement of any of the foregoing polynucleotide sequences;
- (b) detecting in the sample an amount of a polynucleotide that 25 hybridizes to the oligonucleotide;
 - (c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and
 - (d) comparing the amount of polynucleotide detected in step (c) to the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

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- 52. A method according to claim 51, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a polymerase chain reaction.
- 53. A method according to claim 51, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a hybridization assay.
 - 54. A diagnostic kit, comprising:
 - (a) one or more antibodies according to claim 11; and
 - (b) a detection reagent comprising a reporter group.
 - 55. A kit according to claim 54, wherein the antibodies are immobilized on a solid support.
 - 56. A kit according to claim 54, wherein the detection reagent comprises an anti-immunoglobulin, protein G, protein A or lectin.
- 57. A kit according to claim 54, wherein the reporter group is selected from the group consisting of radioisotopes, fluorescent groups, luminescent groups, enzymes, biotin and dye particles.
- 58. An oligonucleotide comprising 10 to 40 contiguous nucleotides that hybridize under moderately stringent conditions to a polynucleotide that encodes a protein, wherein the protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs: 1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317 or a complement of any of the foregoing polynucleotides.

- 59. A oligonucleotide according to claim 58, wherein the oligonucleotide comprises 10-40 contiguous nucleotides recited in any one of SEQ ID Nos: 1, 3-26, 28-77, 142, 143, 146-152, 154-166, 168-176, 178-192, 194-198, 200-204, 206, 207, 209-214, 216, 218, 219, 221-240, 243-245, 247, 250, 251, 253, 255, 257-266, 268, 269, 271-273, 275, 276, 278, 280, 281, 284, 288, 291-298, 301-303, 307, 313, 314, 316 and 317.
 - 60. A diagnostic kit, comprising:

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- (a) an oligonucleotide according to claim 59; and
- 10 (b) a diagnostic reagent for use in a polymerase chain reaction or hybridization assay.

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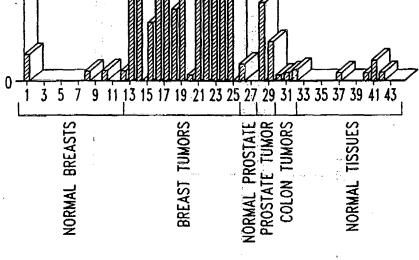
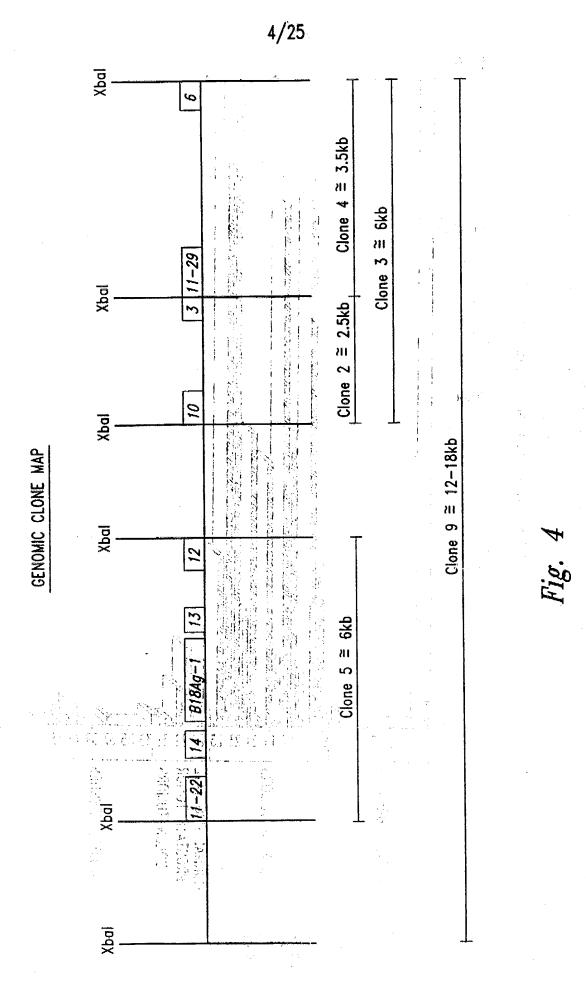
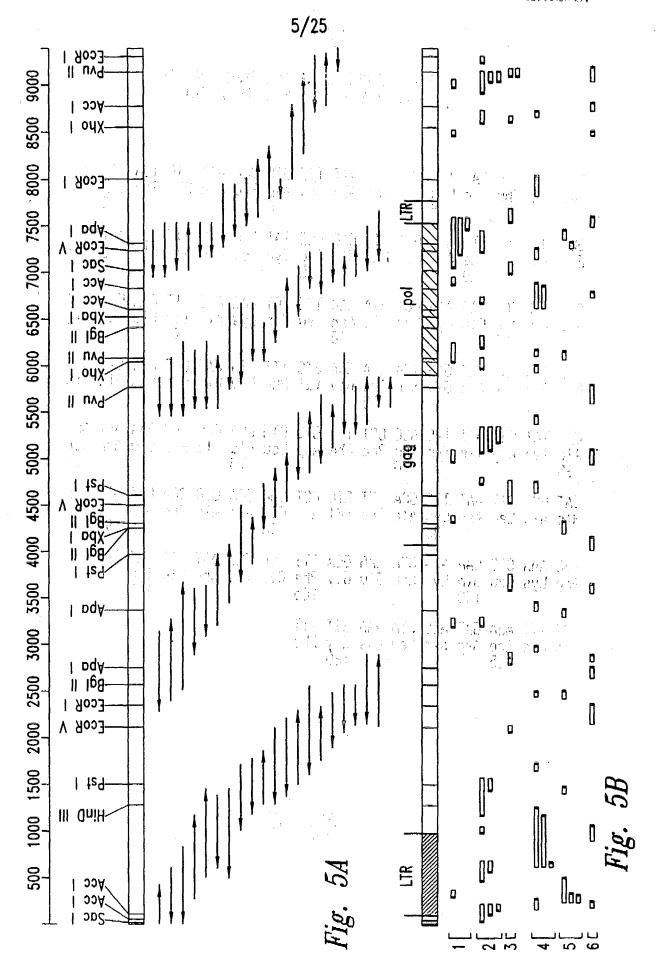


Fig. 3

SUBSTITUTE SHEET (RULE 26)



SUBSTITUTE SHEET (RULE 26)



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NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE BREAST-TUMOR SPECIFIC cDNA B18Ag1

TTA Leu 1	GAG Glu	ACC Thr	CAA Gln	TTG Leu 5	GGA Gly	CCT Pro	AAT Asn	TGG Trp	GAC Asp 10	CCA Pro	AAT Asn	TTC Phe	TCA Ser	AGT Ser 15	GGA Gly	48
GGG Gly	AGA Arg	ACT Thr	TTT Phe 20	GAC Asp	GAT Asp	TTC Phe	CAC	CGG Arg 25	TAT Tyr	CTC Leu	CTC Leu	GTG Va l	GGT Gly 30	ATT Ile	CAG Gln	96
GGA Gly	GCT Ala	GCC Ala 35	CAG Gln	AAA Lys	CCT	ATA I le	AAC Asn 40	TTG Leu	TCT Ser	AAG Lys	GCG Ala	ATT Ile 45	GAA Glu	GTC Val	GTC Val	144
CAG Gln	GGG Gly 50	CAT His	GAT Asp	GAG Glu	TCA Ser	CCA Pro 55	GGA Gly	GTG Val	TTT	TTA Leu	GAG Glu 60	CAC	CTC Leu	CAG Gln	GAG Glu	192
GCT Ala 65	TAT Tyr	CGG Arg	ATT	TAC Tyr	ACC Thr 70	CCT Pro	TTT Phe	GAC Asp	CTG Leu	GCA Ala 75	GCC Ala	CCC Pro	GAA Glu	AAT Asn	AGC Ser 80	240
CAT His	GCT Ala	CTT Leu	AAT Asn	TTG Leu 85	GCA Ala	TTT Phe	GTG Val	GCT Ala	CAG Gln 90	GCA Ala	GCC Ala	CCA Pro	GAT Asp	AGT Ser 95	AAA Lys	288
AGG Arg	AAA Lys	CTC Leu	CAA Gln 100	AAA Lys	CTA Leu	GAG Glu	GGA Gly	TTT Phe 105	TGC Cys	TGG Trp	AAT Asn	GAA Glu	TAC Tyr 110	CAG Gln	TCA Ser	336
GCT Ala	TTT Phe	AGA Arg	GAT Asp	AGC Ser	CTA Leu	AAA Lys	GGT Gly 120	TTT Phe				:				363

NUCLEOTIDE SEQUENE OF THE REPRESENTATIVE BREAST-TUMOR SPECIFIC CDNA B17Ag1

GC	TGGGCACAGT	GGCTCATACC TGTAATCCTG ACCGTTTCAG AGGCTCAGGT	60
CG	CTTGAGCCCA	AGATTTCAAG ACTAGTCTGG GTAACATAGT GAGACCCTAT	120
AA	AAATAAAA	ATGAGCCTGG TGTAGTGGCA CACACCAGCT GAGGAGGGAG	180
	AGGAGA		196

NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE BREAST-TUMOR SPECIFIC CDNA B17Ag2

GC	TTGGGGGCTC	TGACTAGAAA	TTCAAGGAAC	CTGGGATTCA	AGTCCAACTG	6
AC	TTACACTGTG	GNCTCCAATA	AACTGCTTCT	TTCCTATTCC	CTCTCTATTA	120
AA	GGAAAACGAT	GTCTGTGTAT	AGCCAAGTCA	GNTATCCTAA	AAGGAGATAC	180
ΑT	TAAATATCAG	AATGTAAAAC	CTGGGAACCA	GGTTCCCAGC	CTGGGATTAA	2,40
CA	AGAAGACTGA	ACAGTACTAC	TGTGAAAAGC	CCGAAGNGGC	AATATGTTCA	300
TT	GAAGGATGGC	TGGGAGAATG	AATGCTCTGT	CCCCCAGTCC	CAAGCTCACT	360
CT	CCTTTATAGC	CTAGGAGA				38

NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE BREAST-TUMOR SPECIFIC cDNA B13Ag2c

				TTTATTAACC		60
					ATAATTGATC	
					TTTTTATTIC	180
					TTTCTGTAGC	240
					CGTGGGAGAC	300
СТ	ATTTTTTCCA	TATTTGGGCA	ACTACTA	M (ATT)	German Lydyn (1	337

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NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE BREAST-TUMOR SPECIFIC cDNA B13Ag1b

GC	CATACAGTGC	CTTTCCATTT	ATTTAACCCC	CACCTGAACG	GCATAAACTG	60
GC	TGGTGTTTT	TACTGTAAAC	AATAAGGAGA	CTTTGCTCTT	CATTTAAACC	120
ĄΤ	TTCATATTTT	ACGCTCGAGG	GTTTTTACCG	GTTCCTTTTT	ACACTCCTTA	180
TT	TAAGTCGTTT	GGAACAAGAT	ATTTTTCTT	TCCTGGCAGC	TTTTAACATT	240
TT	төтөтегеде,	GGACTGCTGG	TCACTGTTTC	TCACAGTTGC	AAATCAAGGC	300
CC	AAGAAAAAA	AATTTTTTG	TTTTATTTGA	AACTGGACCG	GATAAACGGT	360
CG	GCTGCTGTAT	ATAGTTTTAA	ATGGTTTATT	GCACCTCCTT	AAGTTGCACT	420
GG	GGGGNTTTTG	NATAGAAAGT	NTTTANTCAC	ANAGTCACAG	GGACTTTTNT	480
NA	CTGAGCTAAA	AAGGGCTGNT	TTTCGGGTGG	GGGCAGATGA	AGGCTCACAG	540
TC	TCTTAGAGGG	GGGAACTNCT	A			571

NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE BREAST-TUMOR SPECIFIC CDNA B13Ag1a

TA	ATAACTTAAA	TATATTTTGA	TCACCCACTG	GGGTGATAAG	ACAATAGATA	60
TT	TCCAAAAAGC	ATAAAACCAA	AGTATCATAC)	CAAACCAAAT	TCATACTGCT	120
CC	GCACTGAAAC	TTCACCTTCT	AACTGTCTAC	CTAACCAAAT	TCTACCCTTC	180
66	TGCGTGCTCA	CTACTCTTTT	unung.	TTTNTTTTGG	AGATGGAGTC	240
CA	GCCCAGGGGT	GGAGTACAAT	GGCACAACCT	CAGCTCACTG	NAACCTCCGC	300
TT	CATGAGATTC	TCCTGNTTCA	GCCTTCCCAG	TAGCTGGGAC	TACAGGTGTG	,3 60
TG	CCTGGNTAAT	CTTTTTTNGT	TTTNGGGTAG	AGATGGGGGT	TTTACATGTT	420
TG	GTNTCGAACT	CCTGACCTCA	AGTGATCCAC	CCACCTCAGG	CTCCCAAAGT	480
TA	CAGACATGAG	CCACTGNGCC	CAGNCCTGGT	GCATGCTCAC	TTCTCTAGGC	540
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Fig. 11

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NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE BREAST-TUMOR SPECIFIC CDNA B11Ag1

TG CACATGCAGA ATATTCTATC GGTACTTCAG CTATTACTCA TTTTGATGGC 60 AG CCTATCCTCA AGATGAGTAT TTAGAAAGAA TTGATTTAGC GATAGACCAA 120 GC ACTCTGACTA CACGAAATTG TTCAGATGTG ATGGATTTAT GACAGTTGAT 180 GA GATTATTAAG TGATTATTTT ÄAAGGGÄATC CÄTTAATTCC AGAATATCTT. **∂240** TC AAGATGATAT AGAAATAGAA CAGAAAGAGA CTACAAATGA AGATGTATCA 300 TA TTGAAGAGCC TATAGTAGAA AATGAATTAG CTGCATTTAT TAGCCTTACAT 360 TT TTCCTGATGA ATCTTATATT CAGCCATCGA CATAGCATTA CCTGATGGGC 420 GA ATAATAGAAA CTGGGTGCGG GGCTATTGAT GAATTCATCC NCAGTAAATT 480 AC AAAATATAAC TCGATTGCAT TTGGATGATG GAATACTAAA TCTGGCAAAA 540 GG AGCTACTAGT AACCTCTCTT TTTGAGATGC AAAATTTTCT TTTAGGGTTT 600 638 CT ACTITACGGA TATTGGAGCA TAACGGGA

NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE BREAST-TUMOR SPECIFIC CDNA B3CA3c

ACTGATGGAT	GTCGCCGGAG	GCGAGGGGCC	TTATCTGATG	CTCGGCTGCC	TGTTCGTGAT	60
GTGCGCGGCG	ATTGGGCTGT	TTATCTCAAA	CACCGCCACG	GCGGTGCTGA	TGGCGCCTAT	120
TGCCTTAGCG	GCGGCGAAGT	CAATGGGCGT	CTCACCCTAT	COTTITICCA	TGGTGGTGGC	180
GATGGCGGCT	TCGGCGGCGT	TTATGACCCC	GGTCTCCTCG	CCGGTTAACA	ссстватьст	240
TGGCCCTGGC	AAGTACTCAT	TTAGCGATTT	TGTCAAAATA	GGCGTG		286

14/25

NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE BREAST-TUMOR SPECIFIC cDNA B9CG1

AG	CAGCCCCTTC	TTCTCAATTT	CATCTGTCAC TACCCTGGTG	TAGTATCTCA	60
CA	TTTTTATAGC	стсстсссть	GTCTGTCTTT TGATTTTCCT	GCCTGTAATC	120
AC	ATAACTGCAA	GTAAACATTT	CTAAAGTGTG_GTTATGCTCA	TGTCACTCCT	180
AA	ATAGTTTCCA	TTACCGTCTT	AATAAAATTC GGATTTGTTC	TTTNCTATTN	240
CA	CCTATGACCG	AA		$\begin{array}{cccccccccccccccccccccccccccccccccccc$	262

15/25

NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE BREAST-TUMOR SPECIFIC cDNA B9CG3

60	GGTTTGAGCT CTCTACTGTG TAAACTCCTA AACCAAGGCC	CAAAGCCAGT	AG
	e di li baya e di kabandan kanda wa daya ke di		
120	GGATTTTTAT TATAAACATG TACCCATGCA AATTTCCTAT		
	THOSE THE THEORETH WHICH SEE MANAGES IN		
180	TACATTTAAA CAATAAAAAT AATCTATTTT TAAAAGCCTA	TATATTCTTC	GA
	GTGTTTAATG AGAGGGTATA AGGTATAAAT CACCAGTCAA		
240	GTGTTTAATG AGAGGGTATA AGGTATAAAT CACCAGTCAA	TTAGGTAAGA	AG
261	A	CCTATGACCG	TG

16/25

NUCLECTIDE SEQUENCE OF THE REPRESENTATIVE BREAST-TUMOR SPECIFIC CDNA B2CA2

GG			CGTTTGGCTG			60
ΑT	AGGAAAATTC	CCAAAGAGGG	AATGTCCTGT	TGCTCGCCAG		120
GG			GACTATTGGN		GGTCTTCTGC	180
CG	NCTTGCNANG					208

NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE BREAST-TUMOR SPECIFIC CDNA B3CA1

GG	GCATGGACGC	AGACGCCTGA	CGTTTGGCTG	AAAATCTTTC	ATTGATTCGT	60
					TTTTNTGTT	
GG	ANAAGGCAAN	GAGCTCTTCA	GACTATTGGN	ATTNTCGTTC	GGTCTTCTGC	180
CG	NCTTGCNANG	ATCTTCAT			Control Control	208

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NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE BREAST-TUMOR SPECIFIC CDNA B3CA2

GG	GCATGGACGC	AGACGCCTGA CGTTTGGCTG AAAATCTTTC ATTGATTCGT	60
		CCAAAGAGGG AATGTCCTGT TGCTCGCCAG TTTTTNTGTT	120
			180
CG	NCTTGCNANG		208

B. Dagagarage

NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE BREAST-TUMOR SPECIFIC cDNA B3CA3

AG	GGAGCAAGGA	GAAGGCATGG	AGAGGCTCAN.	GCTGGTCCTG	GCCTACGACT	60
СТ	GTCGCCGGGG	ATGGTGGAGA	ACTGAAGCGG	GACCTCCTCG	AGGTCCTCCG	120
TC	NCCGTCCAGG	AGGAGGGTCT	TTCCGTGGTC	TNGGAGGAGC	GGGGGGAGAA	180
TC	ATGGTCNACA	TCCC A TEST OF	The State of State			204

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NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE BREAST-TUMOR SPECIFIC CDNA B4CA1

20/25

rc	AGGAGCGGGT AGAGTGGCAC	CATTGAGGGG	ATATTCAAAA	ATATTATTTT	60
TG	ATAGTTGCTG AGTTTTTCTT	TGACCCATGA	GTTATATTGG	AGTTTATTTT	120
CC	AATCGCATGG ACATGTTAGA	CTTATTTCT	GTTAATGATT	NCTATTTTTA	180
ΞA	TTTGAGAAAT IGGTTNTTAT	TATATCAATT	TTTGGTATTT	GTTGAGTTTG	240
60	TTAGTATGTG ACCA		1		264

21/25

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Fig. 21A

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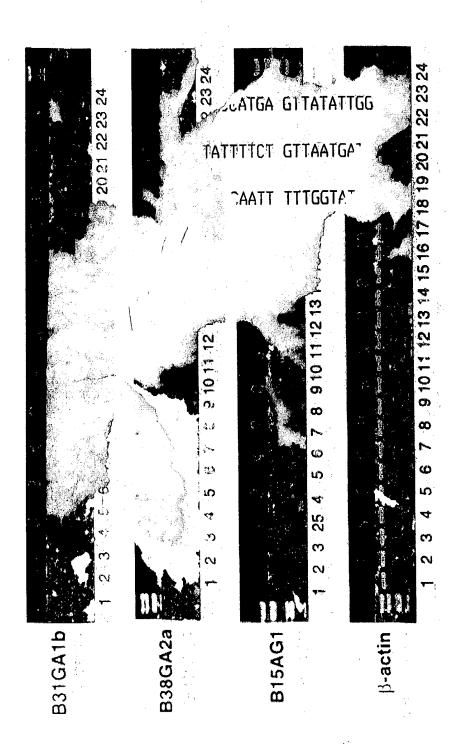


Fig. 21B

Recognition of Peptide by D142 anti-B11-8
CTL line

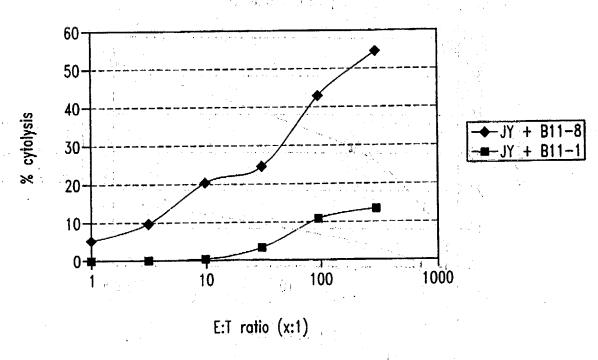


Fig. 22

Recognition of B11 Transductant by B11-8
Specific Clone A1

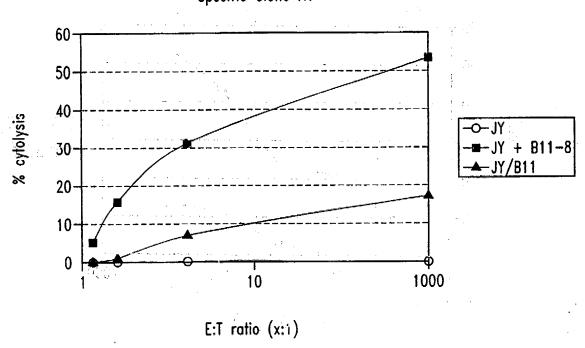


Fig. 23

Recognition of Tumor Cell Lines by Clone A1

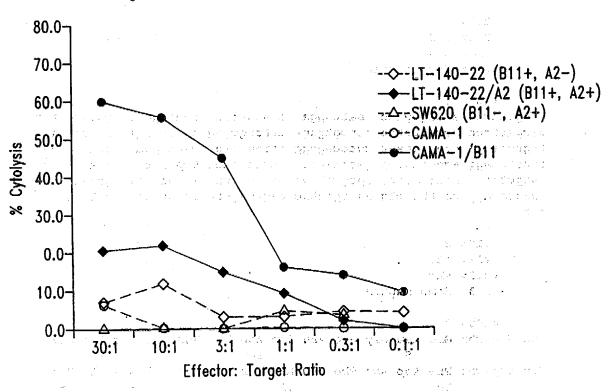


Fig. 24
SUBSTITUTE SHEET (RULE 26)

SEQUENCE LISTING

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<120> COMPOSITIONS AND METHODS FOR THE TREATMENT AND DIAGNOSIS OF BREAST CANCER

<130> 210121.41926PC <140> PCT

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Ala Phe Arg Asp Ser Leu Lys Gly Phe

115 120

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                                                                                                                        120
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                                                                                                                        240
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                                                                                                                         840
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                                                                                                                         900
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aaacneecce etecaaneec eggeenaagn ggaaggttee ettgaateec neeccenena
                                                                                                                        960
                                                                                                                       1020
anggecegga acenttaaan tngtteengg gggtnnggee taaaagneen atttggtaaa
र ४ ५५% वेज्ञानिक अस्ति वृद्धानकोषु वर्षान्यम् राज्यार्थः । सम्बन्धः वर्षान्यम् ।
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                                                                                                                         360
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aaanteetta eecenaaaaa ggttgettag eeceengtee eeacteecee nggaaaaatn
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                                                                    180
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gttttccctt gttggccann atggtctcna acccctgacc tenngtgatc cccccnccn
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nganctenna etgetgggga tnncegnnnn nnncetecen nenennnnnn nenennteen
                                                                    300
tintectine tenninninn enitenitee inettetene enintitint eniconeeni
                                                                    360
connected accommunit tenenthern toteconenn antennenna communitar
                                                                    420
constacate atanacant contestatan cetenarant enetacate tatetectea
                                                                    480
ntnnnnnet communict entenenen thectenith nechencee nectenene
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etnotttnnn ennennntee ntneentten nuteenntnn ennentenen nnenttntte

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                                                                                                                           780
tenentaten centeentta etateteeta tateetteee eteneetaet entteneene
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conntituting time count nething contentition tetetreting inniting to
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                                                       Transfer disaported and the company of
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<211> 545

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	√210× 17	a grayfat to the control of the control of
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		(1) · 重要的复数的概念。如700年 (1) Provide (1) (1) (1) (1) (1)
٠.		in the firm and page the constant of the second of the second of
	<220>	ខេត្ត ខណៈ គ្រួ គ្នាស់ ខណៈ ។ ខេត្ត ខេត្តមាន ខេត្ត
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	tcactcttca cctatgaccg aa	262
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	<210> 19	na an a
	·	- 1977年 - 1975年 - 19
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	aactctgaga tatattcttc tacatttaaa caa	
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	/21/ Hollin pabitall	water of the second the

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                                                                                                                                                                                                                                                                                              180
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                                                                                                                                                                                                                                                                                              180
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           gegtgatggt aegetggetg gageattgat ttetggtgee aaggtgg
                                                                                                                                                                                                                                                                                              287
                                                                                                                                                            ាស់ទូរ៉ូក្រស់ មាន ការប្រជាជនសាស់ ប្រសួទនេនៈ សា
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gtcctaaatg atagttgctg agtttttctt ttaactttcc aatcgcatgg acatgttaga	cttattttct	gttaatgatt	nctattttta	180
ttaaattgga tttgagaaat tggttnttat	tatatcaatt	tttggtattt	gttgagtttg	240
acattatage ttagtatgtg acca			1.5	264
.010. 05			Mark the State	
<210> 25 <211> 376			on Machinery Thank, one on the	
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ctgcatctat ncaacccctg caggcaangc tctggaggca gcagttnggg cttccatcca	gatgcagec	cacactcaca	cnagecatet	360
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-			14 / No. 1	
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$\langle 222 \rangle (1) \dots (3/2)$ $\langle 223 \rangle n = A, T, C \text{ or } G$		n Maria Santa Araba da Araba d Araba da Araba da Ar	and the second second	
			## x 25 x x	
<400> 26	. 1	gala di nessi.		·· 60
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ggtcaaggtt gcatgagtca tgátcgcgcc actgcactcc agcctgggtg acagactgag accetgeete aaaagaaaaa gaataggaag tteagaaace etgggtgtgg ngeecagcaa 240 tetgeattta aacaateeet geaggeaarg etgargeage etaagtreaa gagetgetgt 300 retggaggea gnagtaaggg ettecateea geateaeggn caacactgea aaageaeetg 360 tcctcgttgg ta 372 <210> 27 <211> 477 <212> DNA <213> Homo sapien <400> 27 ttotgtocac atotacaagt tttatttatt ttgtgggttt tcagggtgac taagttttto 60 cctacattga aaagagaagt tgctaaaagg tgcacaggaa atcattttt taagtgaata 120 tgataatatg ggtccgtgct taatacaact gagacatatt tgttctctgt ttttttagag 180 tcacctetta aagtecaate ecacaatggt gaaaaaaaaa tagaaagtat ttgttetace 240 tttaaggaga ctgcagggat tctccttgaa aacggagtat ggaatcaatc ttaaataaat 300 atgaaattgg ttggtettet gggataagaa atteceaact cagtgtgetg aaatteacet 360 gactttttt gggaaaaat agtcgaaaat gtcaatttgg tccataaaat acatgttact 420 attaaaagat atttaaagac aaattettte agagetetaa gattggtgtg gacagaa 477 ការការដំណូន ស្រីគេស្ត្រាមួយ ទីគឺ១៦ សេត្តភេកភេស <210> 28 <211> 438 <212> DNA <213> Homo sapien <220> <221> misc feature <222> (1) ... (438) <223> n = A,T,C or G<400> 28 60 tetneaacet ettgantgte aaaaacettn taggetatet etaaaagetg aetggtatte attecageaa aateceteta gtttttggag ttteetttta etatetgggg etgeetgage 120 180 cacaaatgcc aaattaagag catggctatt ttcgggggct gacaggtcaa aaggggtgta aatcogataa geéteétőga ggtgetétaa aaacacteet ggtgacteat catgeceetg 240 300 gacgacttea ategnettag acaagtetat aggtttetgg geageteect gaatacceae gaggagatae eggtggaaat egfcaaaagt teteeeteea ettgagaaat ttgggteeea 360 420 attaggtece aattgggeet etaateaeta tteetetage tteeteetee ggnetattgg ttgatgtgag gttgaaga 438 <210> 29 <21.1> 620 <212> DNA <213> Homo sapien <220> <221> misc_feature <222> (1) . . . (620) $\langle 223 \rangle$ n = A,T,C or G <400> 29 60 aagagggtac cagccccaag ccttgacaac ttccataggg tgtcaagcct gtgggtgcac 120 agaagteaaa aattgagetti tõggateete ageetagatt teagaggata taaagaaaca 180 cctaacacct agatattcag acaaaagttt actacaggga tgaagctttc acggaaaacc

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240
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                                                                                                                                                                                                                                                                                                          360
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nanqaaatcn ttttaanact tccacggttn aatgactgcc ctattanatt cngaacttan
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atconggect gtgacctett tgetttggec atteceeett tttggaatgg etntttttt
                                                                                                                                                                                                                                                                                                           600
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                                                                                                                                      April San Commence
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tttttttt ttttttt ttttttt
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                                                                                                                         and the true spin stoping digital gight of the region.
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<222> (1)...(762)
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gagtcagcta attaggagag cagagtttag acagcagtag gcaccccatg atacaaacca
                                                                                                                                                                                                                                                                                                            180
                                                                                                                                                                                                                                                                                                            240
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 ctgcccataa aagatggaga gcaggagtgc catccacatc aacacgtgtc caagaaagag
                                                                                                                                                                                                                                                                                                            300
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                                                                                                                                                                                                                                                                                                            360
 aaattagatt tttctctaca tatatataat atacagatat ttaacacatt attccagagg
                                                                                                                                                                                                                                                                                                            420
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  teteaaatte tgaagtatat cagaatggga caggeaatgt tttgeteeac actggggeac
  agacccaaat ggttctgtgc ccgaagaaga gaagcccgaa agacatgaag gatgcttaag
                                                                                                                                                                                                                                                                                                            600
 gggggttggg aaagccaaat tggtantatc ttttcctcct gcctgtgttc cngaagtctc
                                                                                                                                                                                                                                                                                                            660
  cnetgaagga attettaaaa ceetttgtga ggaaatgeee cettaccatg acaantggte
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  ccattgettt tagggngatg gaaacaccaa gggttttgat cc
                                                                                                                                                                                                                                                                                                            762
                            <210> 32
                                                                                                             of the first of the second solder and its face to about
                            <211> 276
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                                                                                                                                                                                                                                                                                                            180
   toaccqaata cootttotaa gaaacgtgtg otgaatgagt gcatggataa atcagtgtot
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                                                                                                                                                                                                                                                                                                            276
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ataacttttt caccgtaage teteetgett gttagtgtag tgtggttata ttaaactttt
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caageceatt atettttte ceceegaaat etgaaaattg caggggacag agggaagtta
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                                                                                                                                                                                            420
aattgtgttt acttgagctg ctgattgtaa gcagttttat ctcaggggca actacta
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                                                                                                                                                                                            360
actgtactgt ttgccattat tacagtcgta caagtgcatg tcaagtcacc cactctctca
                                                                                                                                                                                            420
ggeatcagta tecacetcat agetttacac attttgacgg ggaatattgc ageatcetca
                                                                                                                                                                                            480
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                                                                                                                                                                                            540
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agaacceggea cegattetata ggeaactact a
                ກຣອຣລຣກມະຕາ ພວກພະ ທະສວນ ອຸປະສອດຊີນ ຄວາມ ໄດ້.
≷210> 35
               ម របស់ស្រួនស្នាន់ បានជាសុខ បាន បានប្រ
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                                                                                                                                                                                            540
ttagggcagg tgttcgaaac cagcctgggc aactacta
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                                                                                                                                                                                                                                                                480
    taattccagt aacacggcct gtatacgtct ggtancccta gngaaga
                                                                                  en la livre apparat a ma langap apparte a lighte a la persola de d
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                                                                                                                                                                                                                                                                240
                                                                                                                                                                                                                                                                300
     atttacggtt caaaagaagt tgtaatattg tgcttggaac acagagaacc agttattaac
                                                                                                                                                                                                                                                                331
     ttcctactac tattatataa taaataataa c
                           <210> 44
                                                                              AN AD ADEAN OF A SMITH MARK THERETON STREET STATE CONT.
                           <211> 592
                                                                                人名 化二甲基基 化复数 说:"是$P$ 12 4 4 25 1 美国魏国的大海峡 $P$ 12 4 5 1 1 1 1 1 5
                           <212> DNA
                                                                                                       30、1、2000年1月本,10年1度10gm0477,1207年1月4月1日第中省十月海17日4日
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                                                                                                              人名英格兰 医人名西格尔克斯 电电子 化基础 化拉维尔克 人名意大利特
                                                                                                                                is a constant of the property of the property of the constant 
                           <220>
                                                                                                       Special Control of the Magic Manager Control of the Con-
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                                                                                                                                                                                   Control of the state of the sta
                           <222> (1) ... (592)
                                                                                                          the control of the Admin
                           \langle 223 \rangle n = A,T,C or G
                                                                                                           payment by a layer group which the warms
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     cagagtgacc cttgaggaga tgtgctacac tagaaaagaa ctgcttgagt tttctaattt
                                                                                                                                                                                                                                                                180
                                                                                                                                                                                                                                                                240
      atataagcag aaatctggag aagagtcata ggaatggata ttaagggtgt gagataatgg
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      ttctgctttt gatgttgcag ctcagggagt taaaaaaggt tttaatggtt ctaatagttt
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                                                                                                                                                                                                                                                                420
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ttatactgtg aggaatgáta gccaagggtg gggactttaa gactaaggtg gtttgtactt
                                                                                                                              420
gegeegatga teecaggeag aaagametga tegetagttt tataegggea actaetaage
                                                                                                                              480
cgaattccag cacactggcg gccgttacta attggatccg anctcggtac cagcttgatg
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catascitga gitwictata nigicno
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ttaagetteg ggttggtatg tggtgggaat tgtgagegga taacaattte acacaggaaa
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cagctatgac catgattacg ccaagctatt taggtgacat tatagaataa ctcaagttat
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gcatcaaget tggtacegag tteggateea etagtaaegg eegeeagtgt gtggaatteg
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gettagtagt tgeegaceat ggagtgetae etaggetaga atacetgagy teeteeetag
                                                                                                                              420
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tggacwatcg ataaattaat cctgatagga tgatagcagc agattaatta ctgagagtat
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gttaatgtgt catcectect atataacgta tttgcatttt aatggagcaa ttctggagat
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                                                                                                                              660
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                                                                                                                              780
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                                                                                                                              840
agetgtttee tgtgtgaaat tgttateege teecaattee eeecaccata egageeggaa
                                                                                                                              900
cataaagt
                                                                                                                              908
                        and the second of the second of the
           <2105 47: 1 THE LIMIT TOWN, 1 FOR THE PROPERTY OF THE PROPERT
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ctttaatttc tggaacctag gtctccccat cttcttctgt gctgaggaac ttcttggaag
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cggggattet aaagttettt ggaagacagt ttgaaaacca ccatgttgtt ctcagtacct
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                                                                                                                                                                                                                                                                              420
attatttacc atgccaytar scacatgctc tttgatgggc nyctccstac cctccttaag
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                                                                                                                                                                                                                                                                              120
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tegtgacaat geetateaac ttegtegtea ataagttgtg gaeetteega aeggtgaage
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agecgaatte cageacactg geggeegtta ctaattdgat ecgaacteeg taaccaagee
tgatgcgtaa cttgagttat tctatagtgt ccctaaaata acctggcgtt a
                                                                         Property of the Property of the Community of the Communit
                       <210> 49
                                                                                    ing the control of the companyors of the probability of the form
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 catcacagag ttttcctttt tttttttttg agacagagtc ttgctctgtc acccaggctg
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gaagwatgca aattaaaacc ataatgagaa accactatgt cccactagaa tagataaaat
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                                                                                                                                                                                                                                                                                                                                      120
ggccataccc tgagggaggg gagggatete tagtgttgte agaageggaa getea
                                                                                                                                                                                                                                                                                                                                      175
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                                                                                                  人民大 化工作工作 化二氯化氯化 化基环 化基套流量 化磷酸合物 网络心态 化自动运动器 跨越
                            <211> 223
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                                                                                                                                                                                                                                                                                                                                      120
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 indification of the complete extending a finite of the complete of the complet
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  agtggcagac actgaaaata aggagaatga agttgaagag gtaaaagagg agggtccaaa
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 agagatgact ttggatgggt ggtaaatggc tall a see a se
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	<211> 20		*		•		
	<212> DN		_	•		. 1	
	<213> HO	mo sapie	n.			in the second	
	400 50	•			3.1		•
	<400> 59			h t	<u> </u>		<i>c</i> 0
				tcatcaacca			. 60
				acttcatgct			_
	jtgacgga t	gtggaagc	c acacgtgag	ia craraara	g tgeetegaa	c ctgcccatgt	-
180					ordinal Albertain	, to some of the second second	200
cagto	gatcat tat	gggrygr	aaatggct				208
			-			*	
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	<211> 17	_				Section 2	
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agece	ectoac acc	eattace	aatagettir	tecttetta	acctettaga	gtatttatgg	120
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	egecae aca				3-3-3	To a second	
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					2.1		
	<220>						
	<221> mi	sc featu	re				
	<222> (1	.) (134)	Name and the same of the	4.7 14.1 To 17 N. L.	the state of the s	. <i>i</i>
	<223> n	= A, T, C	OL G.		om grander of the		
	: (.	DD FFTE				e v ° π	
	<400> 61					,	
				ccactcnact			60
actg	gtgaan atg	jteetean	gaaaancncc	acacgcngct	cagggtgggg	tgggaancat	120
cana	atcatc ngg	tc .					134
					1 1 .	. *	
	<210> 62	2			•		
	<211> 14	15	. s≢ysias i sec., († .		44	e de la companya de La companya de la co	
				na dia kaominina dia kaomi Kaominina dia kaominina di			
	<213> Ho	omo sapie	:11	en de la companya de La companya de la co		- 1	
·						." 	
	<400> 62						
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ccaa	geteet tac	etggtacc	ctctt				145
					till state of the second		
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1.44				er en			
13.222				344 844	and the second second	the second second second second	60
_						tgcagcctaa atggtccctc	120
			SECOND CONTRACTOR	augusticidi			

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atcttttgtg agactctact	catagtgtga	taagcactgg	grrggraagg	caagaggagc	300
<210> 65	•				÷.
<211> 203		•		State of the state of	
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ctcatgggtc tctctgctcc	agttctgaac	ctttctcttt	tcctagaaca	tgcatttarg	180
tcgatagaag ttcctctcag					203
	and a second of the	agatara a s	erth takinin nin	a single	
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<211> 344				Parameter State of the	
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(213) Homo Sapid	211				
· <400> 66				1. 500.1	with the state of
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 gtengnaata ggggencata actacagaaa tgcantteat actgetteea ntgecateng
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 cgtgtggcct tncctactct tcttntattc caagtagcat ctctggantg cttccccact
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                                                                                                                                           323
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                                                 CM of Chicarda Chicarda Carres
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  aagacggaac tecaccettt gettggtett aagtatgtat ggaatgttat gataggacat 300
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                                                                                             Ş. M., -
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                            <221> misc_feature ___ag __reads to a garden to a contract the contract to
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   actaaactgt tetteatana acageceata ttattateaa attaagagae aatgtattee
                                                                                                                                              240
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    aatatccttt anggccaata tatttnatgt cccttaatta agagctactg tccgt
               <21.1> 406 . Apprintagio ligitarità più accidente la
               <212>:DNA Romatical graph of condensation of the control of the co
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                                                                                                                                               180
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    tgcgtgtgag catgagtgat ggctagtgtg actgcatgtc agggagtgtg aacaagcgtg
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                                                                                                                                               300
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atgtatectt gteagtaage tatgatgtae agggaacaet geecaaggae acagatattg
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                                                                                                                                                                                                                 402
  cactgaaatc tgagtgttga tcatcacact gctcgactta ca
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                    <211> 193
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                                                                                     The transfer of the property of the contract o
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Cars nome papien	e. 100 - 1 den Saat til det ekki		a, Adio Colombia (New York)	
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        gggactatte teaggetgaa gaaggtggga ggggagggeg gaacergagg agecacetga
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                                              seks titat grover og skilitisk som i det formulåren i en storikelige for en storik til som en storik for som e
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gtgaaaggca tateetettg tetataetga ataceacaag taceettttg accatgiega
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                                                                                                                                       720
tteattttte aggeaaggtg aactgttttg ectataataa emteatetee tgataemega
                                                                                                                                       780
aacceckgga retateaaac cateateate cagegitekt watgtymeta aatecetatt
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                                                                                                                                       960
attgtttccg cccccnttcc ccnccttnna accggaaacc ttaattttna accnggggtt
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                                                                                                                                     1027
cctatcc
                                                                                                        A Commence of the
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                                                        2000年1月1日 - 1987年1月2日 1月2日 - 1987年1月1日 - 1月1日 -
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                                                                                                                                       207
tatactacca gcgtcgtaat gtcacta
            <210> 201
            <211> 209
                                                                                           医皮肤结合 斯森 人名英格兰
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<211> 349				· `&
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tcaaacggca ttgggttata taccatcagc	tgaacttcac	acacatctcc	ttgaacccac	300
tggaaatcta ttttcttgtt ccgctcttct	ccacagtgtt	gcagcgtaa	* **	349
210-203 675.200,00. 74455	omale books something			
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<211> 241 <212> DNA			and the second s	
<213> Homo sapien				
* 4005 203 to the period of personal	. F. (1)			
A TOO A STAN AND A STA	A 18 .			
tgctcctctt gccttaccaa cccaaagccc				60
cagttttcaa cgcaatatag tatagtttat				120
acaactgcta ccaccaccac caacctaggg				180
ttctcctttg agtttcaggc tcctctggga	eccetgica	ccaacgggcg	gtaaatggct	240
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and the second of the second o				
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gagtteettt taettttttt aacettteet ta	
ggggtaataa tgacttgttg gttgattgta ga	
ttttaatctg acgcaggett atgcggagga ga	
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gggatttttt aggtagtggg tgttganctt ga	
rgcctactat gggtggtaaa tggct	505
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gacagactat tctctggaga aaaataaaat gg	
ggccgggcat ggtagcacac acctgtaatc co	and a set of the set o
<210> 207	and the second s
	All Communication of the Commu
	, de l'internation de l'églique d L'églique de l'églique de l'égli
<213> Homo sapien	est for evidential between the right of the second
en e	La Son Chipa - grand Grigoria de la comercia
<400> 207	ella i figuri fagus graniferra en la comulgio. O la campata campa mandre, primo la comuna del
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agactggtac tggtcagtgg cctgggggtt gg	ggacctct attatatggg atacaaattt 120
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<210> 208	
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	1. C - C - C - C - C - C - C - C - C - C
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agctgtaagg catgaaggat gccaagaagt tt	aaggaata tgggtggtaa atggctaggg 180
gacatgagtc agtcta	196
	The Award Name of States and Stat
<210> 209	·
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	a Physical Conference
<220>	en e
<221> misc_feature	et an art
<222> (1) (345)	e i wakili Wanasan ji ikuna a likuk
$\langle 223 \rangle$ n = A,T,C or G	
	l a
	5 700 Billion 1 8 8 1 2 2

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`;	gatttagage aaatttetta tteteettge eteatetgta acatggggat aataatagaa	240
	ctggcttgac aaggttggaa ttagtattac atggtaaata catgtaaaat gtttagaatg	300
: .		345
f	gtgccaagta tctaggaagt acttgggcat gggtggtaaa tggct	343
	210 210	
	CALLY 1/0 TO A CONTROL OF PROPERTY AS A CONTROL OF A CONT	4
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                <210> 214
               <211> 345
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               <221> misc_feature
                                                                       化多种环状 医神经坏 化二甲基胂二苯甲二磺胺甲二甲二甲二
               <222> (1) . . . (345)
                                                                       Entry Souther Being weeking the parties of the
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                                                                        TO TOTAL BUNGS OF SAME DOES TO THE FOLLOWING
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<400> 214
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                                                                                                                                                                                        120
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                                                                                                                                                                                        180
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               <211> 429
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Indipenting di pada garapatan Agarahasa pada asalah mengalah pada kalaman
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                                                                                                                                                                                        180
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tteetateat tgtgaageag aatteaceaa gegttggatt gtteaceeae taatagggaa
                                                                                                                                                                                        300
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                                                                                                                                                                                        360.
ttgccatggt aatcctgctc agtacgagag gaaccgcagg ttcasacatt tggtgtatgt
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gcttgcctt
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                                                                         Control of the Control of the
                                                                          the control of the second of the control of the con
               <220>
                                                                         the section of the district of seedings for the projection
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               <222> (1) . . . (593)
                                                                                                  of graphers in the control of the con-
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ttgccctatg acctcatece tgttccatgq_cc	tattetga titetggtga	actttggage .	360
ageetggttt nteeteetea etceageete te			420
cacncaaang gtcaggtgtg tctggggaat cc			480
tettaaaaac ettettgeet aateanatng tg		ccomangeac	530
coccadada occorrect advantaring og	cccagegg ccaaceneen		330
-210- 220			
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ctgtgtttct gctggaaaag gagggaagag ga	atggctga tttttaccta	atgtctccca	180
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tottgotcag agagoaggto totttaaaac to	agaaggga gaatgagcaa	atgattaaag	300
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<210> 221		ant warm	
<211> 530			
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\(\frac{1}{2}\)	4 - 44 M. M	Tell the State of	
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acaatggggg cacctcctga gaaacacatt gt			300
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cctgaacagc atgggactgt actgaatact gg	• •		420
tatctaaaca cagagaaggt acagtaagaa ta			480
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.000			
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                                                                         240
gagtggattt acacacctca ggagaccact acgtcgatac a
                                                                         281
                      .. ೯೬೮ ಕಲ್ಪಡೆಗಳು
- ೧.೩ ಕರ್ಯಾತ್ಮಾತ್ಮನಿಕರ್ ಅಮ್ಮಿ ವಿಶ್ವದೆಯಾಗು ೧೯೨೦ ಗಳುಕು
- ೧.೩ ಕರ್ಯಾತ್ಮನಿಕರ್
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                                          海南山 医二氯化氯酚 權 "想要是你不管" 化二十九年
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                                                        ្រុំ ខែស្ថិតនៅទៀតនៅគ្នាសាលាបានប្រធានប្រជាជាក្រុម ខេត្តបានប្រធានប្រជាជាក្រុម ខេត្តបានប
aagtecatgg taccetetta ( )
                                                       प्राचनकर स्त्र व्यक्तिक व्यक्ति । व्यक्ति व्यक्ति ।
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aaaaatatgg cacttgtgaa		_	and the second s		: 240
tgtgctatta tgatgatgaa					300
aattootgon aatgtttaat					360
cagaaaagtt agcaggtcan			the second second	Fr. a	420
tgtcgagtaa actanaacag	•	_	_	_	480
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		international state of the second sec		Color of the color	492
<210> 239					
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<210> 239 <211> 482 <212> DNA	en geraal (1) oo de geraal		The second section of the second section of the second section of the second section of the second s	an in Europe d in Gunn in Albertaille in Albertaille in Albertaille page and an air	
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<212> DNA

<213> Homo sapien

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aageettgea gttgagatag aggaagggea etgteteetg eetgeeeetg ggaactgaat
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                                                                519
aaaaacttga nggaactcgg agaccactac gtcgataca
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     <212> DNA
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Tibenori guya ikasolot — Timotifi je angemban bas
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                                                                 240
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atattettga caaagetage atagagacag caattttaca caaggtattt tteacetgtt
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                                                                     240
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cactgataca attgatccaa taccagtttt agtctggcat tgaatcaaat cactgttttt
                                                                     180
gttgtataaa aagagaaata tttagcttat atttaagtac catattgtaa gaaaaaagat
                                                                     240
gettatettt acatgetaaa ateatgatet gtacattggt geagtgaata ttactgtaaa
                                                                     300
agggaagaag gaatgaagac gagctaagga tattgaaggt gcccaa
                                                                     346
                    · 重、 图 · 《四·阿尔斯》:第四 · 克斯克· 斯· 电压器 · 多克尔
      <210> 245
                    THE PROPERTY OF STREET, WITH THE PARTY OF STREET
     <211> 521
      ring the first arm to be because the statement of several regular
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                         orang umo ning distractive the leading of the second
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etgtettaag ceaatgacee etgeagatta ttagageaae tgtteteeae aacagtgtaa
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geetettget anaageteag gteeacaagg geagagattt ttgtetgttt tgeteattge
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                                                                     420
gaatcacctg tganatgggt atgettgtte eccantgttg cagatnaaga tattgaangt
                                                                     480
geccaaatea etanttgegg gegeetgean gtecancata t
                                                                     521
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                                     in digital de ambereum a differencia
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120

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                                 ិស្សាន្តិស្សាស្ត្រ ខេត្តស្ថាល់ស្ថិត ១០ ២០១០ ៤០ខ្លួននឹង
           <223> n=A,T,C or G is the Figure . The sequent of the property of the property of the sequence G
           <400> 248 प्रतिकास्ति स्था जनसम्बद्धाः स्टब्स्ट कार्यक्षाः स्टब्स्ट कार्यक्षाः
ttegatacag geaaacatga actgeaggag ggtggtgacg atcatgatgt tgccgatggt
                                                                                                                                              60
ccggatggnc acgaagacgc actggancac gtgcttacgt ccttttgctc tgttgatggc
cctgagggga cgcaggaccc ttatgaccct cagaatcttc acaacgggag atggcactgg
                                                                                                                                            180
attgantccc antgacacca gagacacccc aaccaccagn atatcantat attgatgtag
                                                                                                                                            240
ttcctgtaga nggccccctt gtggaggaaa gctccatnag ttggtcatct tcaacagyat
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ctcaacagtt tecgatgget gtgatgggea tagtcatant taacentgtn tegaa
                                                                                                                                            355
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            <211> 434
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            <213> Homo sapien
            <400> 249
ttggattggt cctccaggag aacaagggga aaaaggtgac cgagggctcc ctggaactca
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aggateteca ggageaaaag gggatggggg aatteetggt eetgetggte eettaggtee

acctggtcct ccaggcttac ca acccgctggc cagaaaggtg ac tggtgaagtc attcagcctt ta aggcatgcaa gcagatgcag at atttggttcc ctcaattccc tg tcagaccaat ccaa	agtggtet (lecaatett (gataatat (ccagggcct gtcctccaaa ccttgattac	cetgggeete aaaaegagaa teggatggaa	caggtccacc gacatactga tggaagaaat caatgggtac	180 240 300 360 420 434
<211> 430			* '		
<212> DNA <213> Homo sapien					
				and the second	
<220> <221> misc_feature <222> (1)(430) <223> n = A,T,C or	rG			• • • • • • • • • • • • • • • • • • •	
<400> 250 tggattggtc acatggcaga ga	A Company of the Comp	A SECTION OF THE RESIDENCE		1 1	60
tcactagtta ttattattta ti	tttatttt	gagatqaagt	ctcgctttgt	ctcccaggct	120
ggagageggt ggtgegatet to	ggetetetg	caacccccgc	toccaacta	attttttgt	180 240
tagcctcgcg ggtagatgga at gtcttcagta gagacagggt to	tegecatgt	tgggcaggct	ggtcttgaac	teetgacete	300
nagtgatetg cectectegg co	ctcacaaag	tgctggaatt	acaggcatgg	gctgctgcac	360
ccagtcaact tctcactagt to	atggcctta	tcattttcac	cacattctat	tggcccaaaa	420 430
aaaaaaaaan			1 - 1 - 4. Tal - 4		150
<210> 251				\$ 19 c	
<211> 329			13 13 15 15 15 15 15 15 15 15 15 15 15 15 15	Margaret Control	
<212> DNA <213> Homo sapien					
(21) Nome Suprem	•			Mark Sights	
<400> 251					60
tggtactcca ccatyatggg g ggagtctgtg ccgaggtgca g	tcaaccgcc	atcetegeee	rectectage	atccaaaaa	120
tetetgaaga teteetgtaa g	gattetaga	tacaccttta	agatctactg	gategeetgg	180
gtgcgccagt tgcccgggaa a	ggcctggag	tggatggggc	tcatctttcc	tgatgactct	240
gataccagat acagcccgtc c	ttccaaggc	caggtcacca	teteagtega	taägtccatc	300 329
agcaccgcct atctgcagtg g	agtaccaa				343
<210> 252				in Proposition of the State of the Control of the C	
<211> 536			un linea a		
<212> DNA	_				
<213> Homo sapien				Carrier Salar	
<400> 252	o.,	Artist Land Company	Section 18 Section 18	多数,不断成为"成本"。 《新典》的"新典》。	1
tggtactcca ctcagcccaa c	cttaattaa	gaattaagag	ggaacctatt	actattctcc	60
caggetecte tgetetaace a	iggettetgg	gacagtatta	gaaaaggatg	cagaccaaca	120 180
tatgtagate etgtactgge of ttaatggteg ttgagaettg t	cacyaayee	cagetoggat	aggaaaactt	ttgggcagca	240
agaggaagaa ctgcctggaa g	ggggcatca	tgttaaaaat	tacaagggga	acccacacca	300
ggccccttc ccagctctca g	gcctagagta	ttagcatttc	tcagctagag	actcacaact	360
teettgetta gaatgtgeea o	ccggggggag	tecetgtggg	tgatgaggct	ctcaagagtg	420 480
agagtggcat cctatcttct g	grgrgcccac	aggageetgg	coogagaest totootooao	taccaa	536
geology cayyoroge		2000000000			

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and the second of the second o
            <210> 253
                                      gagain in the anner an photograph agnic in the contract of
            <211> 507
                                           Books to State 1978 toward by David Al-
            <212> DNA
                                               anne demonstration and an experience
            <213> Homo sapien
            <220>
            <221> misc_feature
            <222> (1) ... (507)
            \langle 223 \rangle n = A,T,C or G
            <400> 253
ntgttgcgat cecagtaact cgggaagetg aggcgggagg atcacctgag ctcaggaggt
                                                                                                                                                  60
tgaggccgca gtgagccggg accacgccac tacactccag cctggggcat agagtgagac
                                                                                                                                                120
cctccaagac agaaaagaaa agaaaggaag ggaaaagggaa agggaaaagg aaaaggaaaa
                                                                                                                                                180
ggaaaaggaa aaggaaaaga caagacaaaa caagacttga atttggatct cctgacttca
                                                                                                                                                240
attttatgtt ctttctacac cacaattcct ctgcttacta agatgataat ttagaaaccc
                                                                                                                                                300
ctcgttccat tctttacagc aagctggaag tttggtcaag taattacaat aatagtaaca
                                                                                                                                                360
                                                                                                                                                420
aattigaata ttatatgeea ggtgttttte attectgete teacttaatt etcaccacte
                                                                                                                                                480
tgatataaat acaattgctg cogggtgtgg tggctcatgc ctgtaatccc ggcactttgg
                                                                                                                                                507
gagaccgagg tgggcggats gcaacaa
                                                       <210> 254
                                 त्राचनत्रात्रं सेवजन्त्रत् अन्तर्भाष्ट्रभाष्ट्रभाष्ट्रभाष्ट्रभाष्ट्रभाष्ट्रभाष्ट्रभाष्ट्रभाष्ट्रभाष्ट्रभाष्ट्रभ
             en nem skupe en ekonte komer. En kalenger til en for en er elle filter
             <212> DNA
             <213> Homo sapien
             <220>
             <221> misc feature
             <222> (1) ... (222)
             \langle 223 \rangle n = A,T,C or G
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 actggccaca cttlctccrg cogcettect caaagetgaa gacacacaga gcaaggeget
                                                                                                                                                120
                                                                                                                                                 180
 tetgttutae recedentgg taactecaan centagatgg ttagetneec tgeteatett
 tecacatece tgutatteag tatagteege ggaccaatee aa
             <210> 255
             <211> 463
             <212> DNA
             <213> Homo sapien
             <400> 255
                                                                                                                                                   60
 tgttgcgatc cataaatgct gaaatggaaa taaacaacat gatgagggag gattaagttg
                                                                                                                                                 120
 gggagggagc acattaaggt ggccatgaag tttgttggaa gaagtgactt ttgaacaagg
 ccttggtgtt aagagctgat gagagtgtcc cagacagagg ggccactggt acaatagacg
                                                                                                                                                 180
 agatgggaga gggcttggaa ggtgtgcgaa ataggaagga gtttgttctg gtatgagtct
                                                                                                                                                 240
 agtgaacaca gaggegagag gecetggtgg gtgcagetgg agagttatge agaataacat
                                                                                                                                                 300
 taggccctgt gggggactgt agactgtcag caataatcca cagtttggat tttattctaa
                                                                                                                                                 360
 gagtgatggg aagccgtgga aagggggtta agcaaggagt gaaattatca gatttacagt
                                                                                                                                                 420
 gataaaaata aattggtotg gotactgggg aaaaaaaaaa aaa
                                                                                                                                                 463
                                          CONTRACTOR CONTRACTOR CONTRACTOR
```

the party of the contract of the contract of the

<210> 256

<211> 262

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<212> DNA
            <213> Homo sapien
            <400> 256
ttggattggt caacctgctc aactctacyt ttcctccttc ttcctaaaaa attaatgaat
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ccaatacatt aatgccaaaa cccttgggtt ttatcaatat ttctgttaaa aagtattatc
                                                                                                                                                     120
cagaactgga cataatacta cataataata cataacaacc cetteatetg gatgcaaaca
                                                                                                                                                     180
totattaata tagottaaga toactttoad titacagaag caacatootg tigatgitat
                                                                                                                                                     240
tttgatgttt ggaccaatcc aa
                                                                                                                                                     262
             <210> 257
             <211> 461
             <212> DNA
             <213> Homo sapien
             <220>
             <221> misc_feature
             <222> (1)...(461)
             <223> n = A,T,C or G
                                                            Contract the second second
             <400> 257
gnggnnnnnn nnncaatteg actengttee entggtance ggtegacatg geegegggat
                                                                                                                                                       60
taccgettgt nnetgggggt gtatggggga etatgacege ttgtagetgg gggtgtatgg
                                                                                                                                                     120
gggactatga ccgcttgtag mtggkggtgt atgggggact atgaccgctt gtcgggtggt
                                                                                                                                                     180
eggataaace gaegeaaggg aegtgatega agetgegtte eegetettte geateggtag
                                                                                                                                                     240
ggatcatgga cagcaatate egeattegye tgaaggegtt egaceatege gtgetegate
                                                                                                                                                     300
aggegacegg egacategee gacacegeae geegtacegg egegeteate egeggteega
                                                                                                                                                     360
tecegettee caegegeate gagaagttea eggteaaceg tggecegeac gtegacaaga
                                                                                                                                                     420
agtegegega geägttegag gtgegtacet acaageggte a
                                                                                                                                                     461
                                                                                                    . 9.5
             <210> 258
             <211> 332
             <212> DNA
                                                               trograms do distributo en regional isabilità de servicio de la companya de la companya de la companya de la co
             <213> Homo sapien
                                                              , 1999 - Propri Gentrale Later Beginner i Seder by Histories de d
                                                            rumgu inukin artar nu inaki darake banga ili dinterkerki
             <220>
                                                          ARTOTO COLUMN ARRA CATAL EL WERDONADES
             <221> misc_feature
                                                                               TO CONTRACT SANDAR SERVICES OF A SERVICE STATE OF SANDERS OF SANDAR SAND
             <222> (1)...(332)
                                                             <223> n = A,T,C or G
                                                                and the second of the whole supplies to the start
            <400> 258
tgaccgcttg tagctggggg tgtatggggg actacgaccg cttgtagctg ggggtgtatg
                                                                                                                                                      60
ggggactatg accepttgta gctgggggtg tatgggggac tatgaccect tgtagctggg
                                                                                                                                                     120
ggtgtatggg ggactaggac cgcttgtagc tgggggtgta tggggggacta tgaccgcttg
                                                                                                                                                     180
 tagetggggg tgtatggggg actacgaccg ettgtagetg ggggtgtatg ggggactatg
                                                                                                                                                     240
 accectteta neteegeete tatgegeese tatgaceget teteete gegegeteeg
                                                                                                                                                     300
 aggagagttg tggttgggga aaaaaaaaaa aa
                                                                                                                                                     332
             <210> 259
             <211> 291
                                                                                                     $28.45 mb (b) 15.7 (c) 11. 35
             <212> DNA
             <213> Homo sapien
                                                                                                  Strategy by the second
             <220>
             <221> misc_feature
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<222> (1) . . . (291) <223> n = A, T, C or G<400> 259 60 tacegettgt gacegettgt gacegettgt gacegettgt gacegettgt gaccgcttgt gaccgcttgt gaccgcttgt gaccgcttgt gaccgcttgt 120 180 gaccgcttgt gaccgcttgt nacngggggt gtctggggga ctatgannga ntgtnactgg 240 gggtgtctgg gggnctatga nngantgtna cngggggtgt ctgggggact atganngact gtgcnncctg ggggatcnga ggagantngn ggntagngat ggttngggan a 291 <210> 260 <211> 238 <212> DNA <213> Homo sapien <400> 260 taagagggta ctggttaaaa tacaggaaat ctggggtaat gaggcagaga accaggatac 60 tttgaggtca gggatgaaaa ctagaatttt tttctttttt tttgcctgag aaacttgctg 120 ctctgaagag gcccatgtat taattgcttt gatcttcctt ttcttacagc cctttcaagg 180 gcagagccct ccttatcctg aaggaatctt atccttagct atagtatgta ccctctta 238 <210> 261 and the contraction of the contr <211> 746 <213> Homo sapien <222> (1)...(746) $\langle 223 \rangle$ n = A,T,C or G <400> 261 ttgggcacct tcaatatcaa tagctaacat ttattgagtg tttatcgtat cataaaacac 60 tgttctaagc ctttaaacgt actaattcat ttaatgctca taatcacttt agaaggtggg 120 tactagtatt agtotoattt acagatgoaa catgoaggoa cagagaggtt aattaacttg 180 cccaaggtaa cacagctaag aaatagaaaa aatattgaat ctggaaagtt gggcttctgg 240 300 gtaacccaca gagtettcaa tgageetggg geetcactca gtttgetttt acaaagcgaa 360 tgagtaacat cacttaattc agtgagtagg ccaaatggag gtcagctacg agtttctgct 420 gttcttgcag tggactgaca gatgtttaca acgtctggcc atcagtwaat ggactgatta 480 tcattgggaw gtgggtgggc tgaatgttgg ccagtgaagt ttattcawgc catattttta 540 tgtttaggat gacttttggc tggtcctagg gcaagctctg tctgscacgg aacacagaat 600 wacacaggga coccetcaat ttetggtgtg getagaacca tgaaccaetg gttgggggaa caageggtea aaacetaagt geggeegget ggeagggtee acceatatgg ggaaaactee 660 720 cnacgcgttt ggaatgcctn agctngaatt attctaanag ttgtccncnt aaaattagcc 746 tgggcgttaa tcangggtcn naagcc <210> 262 <211> 588 <212> DNA <213> Homo sapien <220> <221> misc_feature <222> (1)...(588)

 $\langle 223 \rangle$ n = A,T,C or G

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tttgtctgtt tettettet etttteette ceatafecte etaatttacg tttgaettgt
                                                                                                                                                                                            120
ttgctgagga ggcaggaget agagactgct gtgageteat aggggtggga agtttateet
                                                                                                                                                                                            180
tcaagtcccg cccactcatc actgcttctc accttcccct gaccaggctt acaagtgggt
                                                                                                                                                                                            240
tettgeetge ttteeetttg gacceaacaa geecetgtaa tgagtgtgea tgactetgae
                                                                                                                                                                                            300
agetgtggae teagggteet tggetaeage tgeeatgtaa aatateteat eeagtteteg
                                                                                                                                                                                            360
caaattgtta aaataaccac atttcttaga ttccagtacc caaatcatgt ctttacgaac
                                                                                                                                                                                            420
tgctcctcac acccagaagt ggcacaataa ttcttgggga attattactt tttttttct
                                                                                                                                                                                            480
ctctnttnnc gnnngnnnng gnnngnccag gaattaccac nttggaagac ctggccngaa
                                                                                                                                                                                            540
tttattatan aggggagccg attntttttc ctaacacaaa gcgggtca
                                                                                                                                                                                            588
                <210> 263
                                                       ENGLISH SHOWERS A CONTRACTOR OF SHIPS A
                <211> 730
                                                     union of the comment of the comment of the com-
                 <212> DNA
                                                                                               Contract to the second of the test
                <213> Homo sapien
                 <220>
                 <221> misc_feature
                 <222> (1)...(730)
                 <223> n = A, T, C or G
                 <400> 263
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                                                                                                                                                                                               60
 agactgcaaa aagattaaat gtaaaagttg tettgtatae agtaatgttt aagataeeta
                                                                                                                                                                                             120
 ttanatttat aaatggaaaa ttagggcatt tggatataca agttgaaaat tcaggagtga
                                                                                                                                                                                             180
 ggttgggctg gctgggtata tactgaaaac tgtcagtaca cagatgacat ctaaaaccac
                                                                                                                                                                                             240
                                                                                                                                                                                             300
 aaatctggtt ttattttagc agtgatatgt gtcactccca caaaagcctt cccaattggc
 ctcagcatac acaacaagtc acctccccac agccctctac acataaacaa attccttagt
                                                                                                                                                                                             360
 ttagttcagg aggaaatgcg cccttttcct tccqctctag gtgaccqcaa ggcccagttc
                                                                                                                                                                                             420
  togtcaccaa gatgttaagg gaagtotgoo aaagaggcat otgaaaggaa ataaggggaa
                                                                                                                                                                                             480
  tgggagtgac cacaaaggaa agccaaggan aaactttgga gaccgtttct aganccctgg
                                                                                                                                                                                             540
                                                                                                                                                                                             600
  catttcacaa caaaactcng gaacaaacct tgtctcatca atcatttaag cccttcgttt
 ggannagact ttctgaactg ggcgctgaac ataancetca ttgaatgtct tcacagtete
                                                                                                                                                                                             660
  ccagctgaag gcacaccttg ggccagaagg ggaatcttcc aggtcctcaa nacagggctc
                                                                                                                                                                                              720
                                                                                                                                                                                              730
  gccctttgnc
                  <210> 264
                  <211> 715
                                                                           grands and the filter of the same of the filter of
                  <212> DNA
                  <213> Homo sapien
                                                                                <220>
                                                                            The state of the s
                  <221> misc_feature
                  <222> (1) ... (715)
                                                                                          ignored section of magginer processing for the following section of the
                  \langle 223 \rangle n = A,T,C or G
                   <400> 264
                                                                                                                                                                                                 60
  ttttttttt tttggccagt atgatagtct ctaccactat attgaagctc ttaggtcatt
   tacacttaat gtggttatag atgctgttga gcttacttct accaccttgc tatttctccc
                                                                                                                                                                                              120
   gtotottttt tgttootttt otottotttt octoocttat tttataattg aattttttag
                                                                                                                                                                                              180
   gattctattt tatatagatt tatcagctat aacactttgt attctttgt tttgtggttc
                                                                                                                                                                                              240
                                                                                                                                                                                              300
   ttetgteatt teaatgtgea tettaaaete atcacaatet atttteaaat aatateatat
   aacettacat ataatgtaag aatetaccae catatattte cattteteee ttecateeta
                                                                                                                                                                                              360
```

tgtntgtcat attititect ttatal ttgcttaaaa tgtgatcaat attic tttattctca aatnnaccta atatt gcattggttt tttccggctt aagaa ctgggagagg aattctccca agctt	ttcaa ngaaacgtaa tccta ccatntctna cctcc tctaaagcac	aaattcaaaa tacntttcaa tctaagcaga	taaatntetg gaatetgaag attaagtett	420 480 540 600 660
ggccgggaaa tagaaattcc aagtt	aacag gntantttt	ntttttnttn	tence	715
<210> 265 <211> 152 <212> DNA	មានម្លៃសមានម្លាស់ នៃប្រាស់ សមានប្រជាពល់ និក្ខាស់ មានប្រកួតសំលាន អាស់ ប ស្រែស់សំពេញ សុសសំព ស្រែស់សំពេញ មានសំព			da la
<400> 265				
ttttttttt tttcccaaca caaag tgattcccat gaagaggtta tgatt ggttaaatct ttttttttg agacg	tctaa agaaaacatg			60 120 152
000, 000				
<210> 266 <211> 193 <212> DNA <213> Homo sapien			Table 1 The Park Co. William Co. W. William Co. W.	
<220>				
<221> misc_feature	ing Maskedak dalah bermulak kal	er graderske i de		
$\langle 222 \rangle$ (1)(193) $\langle 223 \rangle$ n = A,T,C or G	The striking of the second	•	ing the second	
	The state of the s		en Deren der State d Der State der State d	
<400> 266 taaactccgt ccccttctta atcaa	tatoo agotaccoa	ctccacatta	cettettte	60
aagggactgt ttccgtaact gttgt	gggta ttcacgacca	ggcttctaaa	cctcttaaaa	120
ctcccaatt ctggtgccaa cttgg	acaac atgettttt	tttttttt	tttttttt	180 193
gagacggagt tta		San		
<210> 267	in de la Carlon de La carlon de la Car	entra en estado en e En entra en entra en entra entra en en		
<211> 460 <212> DNA		1 - 4 - 5		
<213> Homo sapien			a ·	•
<400> 267				
tgttgcgatc ccttaagcat gggtg				60
atttacgtct tatctttaga gattg ttcttgaatg tcaattccca agtaa				120 180
ttgcagcaag gctacaatgc tatgg				240
geteagagat geeetteace teeca				300
ggtgtttttg gactccctcg atgcc aaaacactga atgctggggc gtact				360 420
tactggcatg acccataaaa ggagg				460
<210> 268			and the second	
<211> 533	Topic Control of the			
<212> DNA	ing Saada			
<213> Homo sapien				
<220>	1.02. 1.05. 3.00. 1.00.			
<221> misc feature	ne teen en		A	

<222> (1) ... (533)

<211> 102

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\langle 223 \rangle n = A,T,C or G
                <400> 268
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                                                                                                                                                                                                    120
acgeceggge gegttegatt taceggaage gegagetgea gtgggettge geeceeggee
                                                                                                                                                                                                    180
aaattetttg gggggtttaa ggeegegggg aatttgaggt atetetatea gtatgtagee
                                                                                                                                                                                                    240
aagttggaac agtcgccatt cocgaaatcg ctttctttga atccgcaccg cctccagcat
                                                                                                                                                                                                    300
tgcctcattc atcaacctga aggcacgcat aagtgacggt tgtgtcttca gcagctccac
                                                                                                                                                                                                    360
tecataaeta gegegetega eetegtette gtaegegeea ggteegtgeg tgegaattee
                                                                                                                                                                                                    420
caactceggt gagttgegea tttcaagttn egaaactgtt egeeteeacn atttggeatg
                                                                                                                                                                                                    480
ttcacgcatg acacggaata aactcgtcca gtaccgggaa tgggatcgca aca
                                                                                                                                                                                                    533
                 <210> 269
                 <211> 50
                 <212> DNA
                 <213> Homo sapien
                <400> 269
ttttttttt ttcqcctgaa ttagctacag atcctcctca caagcggtca
                 <210> 270
                                                                                          The transfer of the transfer of the state of the second
                 <211> 519
                                                            and the control of the control of the second of the control of the
                 <212> DNA
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teaccaggaa tteacacace teacagtaaa cateagactt tgetgggace tegtgettet 360
taatgggete caccagttee agggeaggga tgacattett ggaggecact ttggeggga
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ccttgggttg catgtgcatc atcatctggg atcgcaaca 519
                                                                                                                    <210> 271
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                                                              大量的 化克克克 化二氯基苯甲基磺基酚基苯甲酚
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 gaacagcaca atggcaagac cattttcgcc tactttacgg gttctaagga cgccgggggg
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                                                                                                                                                                                                     240
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                                                                                                                                                                                                     360
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                                                                                                                                                                                                     420
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                                                                                                                                                                                                                                                                       102
                      <210> 273
                      <211> 455
                      <212> DNA
                                                                                                                   organit in the state of the de-
                                                                                                   South of the second sec
                      <213> Homo sapien
                                                                                         g i sanggana di aktoryo telebik i
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                                                                                                                                                                                                                                                                       120
gittaagtet teggeegaag tiaatetege grittiggea ateaacaggi tiaagtette
                                                                                                                                                                                                                                                                       180
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                                                                                                                                                                                                                                                                       300
ggcaatcaag aggittaagi citcggccga agitaatcic gigittiigg caatcaacag
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gtttaagtet teggeegaan ttaatetegt gtttttggea ateaacaggt ttaantette
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                                                                                                                                                                                                                                                                       455
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                      <211> 461
                      <2129 DNA BERBEN, TER TOLDER VER TELEVISION
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                   କ୍ଷ୍ରୌ ବ୍ୟବ୍ୟକ୍ତ । ଏହା ପ୍ରତିଷ୍ଠୀତ ହୁନ୍ଦେ ଅନୁକ୍ରମ୍ୟ ଅନ୍ତର୍ଶ୍ୱ ଅନୁକ୍ରମ ଅନ୍ତର୍ଶ୍ୱ ଅନ୍ତର୍ଶ୍ୱ । ୧୯୮୯ ଓଡ଼ି ଅନ୍ତର୍ଶ୍ୱ
                                                                    company and adapting different sections.
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                                                                                                                                                                                                                                                                       180
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accognetty ngngneanth concetence contyttee etgnngthae astrongthth
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neegeeneee naatteeeae eenaateaea gegaaneeng aaggeetten naagtettta
                                                                                                                                                                                                                                                   540
angeeengng gttteetent ntanttgeag cetaceetee enettnnnnt tnegngttgg
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nnnctnttcc tnnnactage tngcctntcc nencegnggn neanngcaca ttnenennac
                                                                                                                                                                                                                                                   720
tntgtnncc ...
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                                                                                               医大大性 网络萨斯特斯萨莫尔斯克斯斯斯特斯 化聚基氯化 化安全性抗凝胶性
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nccctgctta ggggaatttt taaagaagat ggctctccat gttcanggtc aatcacnaat
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tgcc
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cacactcaca cacgtatgca	acgtggagat	gtcgcyttww	kkktwywcwm	rmrycrwcgn	420
aatcacttan n	e.	*. · · · · · · · · · · · · · · · · · · ·	(x,y) = (x,y) + (x,y)	e many e may,	431
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-400- 202					
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					30
<210> 283					.1.3
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<213> Homo sapier	n				
				• 60	
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tataaactgc tgtatctagg g	ggcaggacca	agggggcagg	ggcaacagcc	ccagcgtgca	120
gggccascat tgcacagtgg a					180
egttttteet gtattatetg (_	240
hacasgatga atccaawggt					300
ttggcggtgg gggcatasgc (_	_		360
cmcttgawtc cncnccttnn i				•	420
natctgcact anctccctcn				and the second s	480
acnececet enectatee		_			
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nggneeacee micecenate i		Connecente	CCCC	At a second	703
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		4 - 4 - 4 - 4 - 4 - 4 - 4 - 4 - 4 - 4 -	
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	David getien		
<210> 288 - Lightens, his paragraph of the		1.5	* *
(4) 1 (211) 199 (日本の日本の日本の内部を関係をしまいた。	State Control Control	19 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 100
e <212> DNA		Parameter Contract	e e
<213> Homo sapien	$(\delta x, x, y, z) = 1, y, z$	And the second	45
	Marie San	$\mathbb{M}_{n}(\mathcal{A}_{n}) = \mathbb{M}^{n}(\mathbb{R}^{n} \times \mathbb{R}^{n})$, . 1
g<400><268 geraters of the control			60
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<210> 291 <211> 1851

<212> DNA

<213> Homo sapien

<400> 291

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<213> Homo sapien

<400> 296

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<210> 297 <211> 1855

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<220>

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45 40 Gln Lys Arg Thr Ala Leu His Leu Ala Ser Ala Asn Gly Asn Ser Glu 55 Val Val Lys Leu Xaa Leu Asp Arg Cys Gln Leu Asn Val Leu Asp **75** (2012) April 10 (110) 70 Asn Lys Lys Arg Thr Ala Leu Xaa Lys Ala Val Gin Cys Gln Glu Asp 95 90 85 Glu Cys Ala Leu Met Leu Leu Glu His Gly Thr Asp Pro Asn Ile Pro 105 100 Asp Glu Tyr Gly Asn Thr Thr Leu His Tyr Ala Xaa Tyr Asn Glu Asp 115 120 125 Lys Leu Met Ala Lys Ala Leu Leu Leu Tyr Gly Ala Asp Ile Glu Ser 130 135

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Lys Asn Lys Val

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<212> DNA

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<213> Homo sapien

<400> 303

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e egusers ud tokket jud <211> 384 A TOTAL AREA STATE TO THE MANY A STATE OF THE SECOND STATES <212> PRT <213> Homo sapien 等的人 医二次联系 地名美国克尔斯 医二种毒素 Met Val Val Glu Val Asp Ser Met Pro Ala Ala Ser Ser Val Lys Lys Pro Phe Gly Leu Arg Ser Lys Met Gly Lys Trp Cys Cys Arg Cys Phe ~ 5 2 25 7 8 9 9 9 9 9 9 9 9 9 30 9 Pro Cys Cys Arg Glu Ser Gly Lys Ser Ası Val Gly Thr Ser Gly Asp 1 1 40 West Professor 145' to the rest of the His Asp Asp Ser Ala Met Lys Thr Leu Arg Ser Lys Met Gly Lys Trp Cys Arg His Cys Phe Pro Cys Cys Arg Gly Ser Gly Lys Ser Asn Val 75 70 Gly Ala Ser Gly Asp His Asp Asp Ser Ala Met Lys Thr Leu Arg Asn 90 Lys Met Gly Lys Trp Cys Cys His Cys Phe Pro Cys Cys Arg Gly Ser 105 Gly Lys Ser Lys Val Gly Ala Trp Gly Asp Tyr Asp Asp Ser Ala Phe 115 () He company 120 () 120 () He company () 125 () He company Met Glu Pro Arg Tyr His Val Arg Gly Glu Asp Leu Asp Lys Leu His 130 februaris (1964 - 135 februaris) (1964 - 140 februaris) Arg Ala Ala Trp Trp Gly Lys Val Pro Arg Lys Asp Leu Ile Val Met Leu Arg Asp Thr Asp Val Asn Lys Lys Asp Lys Gln Lys Arg Thr Ala 165 Leu His Leu Ala Ser Ala Asn Gly Asn Ser Glu Val Val Lys Leu Leu 190 180 Leu Asp Arg Arg Cys Gln Leu Asn Val Leu Asp Asn Lys Lys Arg Thr 195 Ala Leu Ile Lys Ala Val Gin Cys Gin Glu Asp Glu Cys Ala Leu Met 210 220 Leu Leu Glu His Gly Thr Asp Pro Asn Ile Pro Asp Glu Tyr Gly Asn Thr Thr Leu His Tyr Ala Ile Tyr Asn Glu Asp Lys Leu Met Ala Lys Ala Leu Leu Leu Tyr Gly Ala Asp Ile Glu Ser Lys Asn Lys His Gly 260 265 Leu Thr Pro Leu Leu Leu Gly Val His Glu Gln Lys Gln Gln Val Val 275 Lys Phe Leu Ile Lys Lys Lys Ala Asn Leu Asn Ala Leu Asp Arg Tyr 1 10 10 1 25, 2011 24 **295** 44 21 4 2 1 5 Gly Arg Thr Ala neu Ile Leu Ala Val Cys Cys Gly Ser Ala Ser Ile 310 315 Val Ser Leu Leu Glu Gln Asn Ile Asp Val Ser Ser Gln Asp Leu Ser Gly Gln Thr Ala Arg Glu Tyr Ala Val Ser Ser His His His Val 340 Ile Cys Gln Leu Leu Ser Asp Tyr Lys Glu Lys Gln Met Leu Lys Ile 355 Ser Ser Clu Asn Ser Asn Pro Glu Asn Val Ser Arg Thr Arg Asn Lys 375

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<400> 305

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Leu Arg Asp Thr Asp Val Asn Lys Lys Asp Lys Gln Lys Arg Thr Ala
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Asp Asn Lys Lys Arg 145	Thr Ala Leu 150	Thr Lys Ala 155	Val (Gln Cys	
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		Leu His Tyr 185	Ala Val Tyr	
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Ser Lys Asn Lys His 210				
Gln Lys Gln Gln Val 225	Val Lys Phe 230	Leu Ile Lys 235	Lys Lys Ala	Asn Leu 240

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(19) World Intellectual Property Organization International Bureau



(43) International Publication Date 19 October 2000 (19.10,2000)

PCT

(10) International Publication Number WO 00/61753 A3

- (51) International Patent Classification⁷: C12N 15/12, C07K 14/47, 16/18, 19/00, C12N 15/62, A61K 38/17, 39/395, 48/00, C12N 5/08, G01N 33/574, C12Q 1/68
- (21) International Application Number: PCT/US00/09312
- (22) International Filing Date: 7 April 2000 (07.04.2000)
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English

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- (71) Applicant: CORIXA CORPORATION [US/US]; Suite 200, 1124 Columbia Street, Seattle, WA 98104 (US).
- (72) Inventors: FRUDAKIS, Tony, N.; 7937 Broadmoor Pines Boulevard, Sarasota, FL 34243 (US). SMITH, John, M.; 208 - 116th Place S.E., Everett, WA 98208 (US). REED, Steven, G.; 2843 - 122nd Place N.E., Bellevue, WA 98005 (US). MISHER, Lynda, E.; 6251 53rd Avenue N.E., Seattle, WA 98115 (US). RETTER, Marc, W.; 33402 N.E. 43rd Place, Carnation, WA 98014 (US). DILLON, Davin, C.; 21607 N.E. 24th Street, Redmond, WA 98053 (US).

- (74) Agents: POTTER, Jane, E., R.; Seed Intellectual Property Law Group PLLC, Suite 6300, 701 Fifth Avenue, Seattle, WA 98104-7092 et al. (US).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

- With international search report.
- (88) Date of publication of the international search report: 28 June 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: COMPOSITIONS AND METHODS FOR THE TREATMENT AND DIAGNOSIS OF BREAST CANCER



cDNA PREPARED FROM NORMAL BREAST TISSUL FROM THE SAME PATIENT

cDNA PREPARED FROM BREAST TUMOR

- 818Ag1

(57) Abstract: Compositions and methods for the detection and therapy of breast cancer are disclosed. The compounds provided include nucleotide sequences that are preferentially expressed in breast tumor tissue, as well as polypeptides encoded by such nucleotide sequences. Vaccines and pharmaceutical compositions comprising such compounds are also provided and may be used, for example, for the prevention and treatment of breast cancer. The polypeptides may also be used for the production of antibodies, which are useful for diagnosing and monitoring the progression of breast cancer in a patient.

) 00/61753 A3

CLASSIFICATION OF SUBJECT MATTER PC 7 C12N15/12 C07K14/47 C12N15/62 C07K16/18 C07K19/00 G01N33/574 A61K39/395 A61K48/00 C12N5/08 A61K38/17 C12Q1/68 According to International Patent Classification (IPC) or to both national classification and IPC B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) CO7K A61K G01N C12Q C12N Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal C. DOCUMENTS CONSIDERED TO BE RELEVANT Relevant to claim No. Citation of document, with indication, where appropriate, of the relevant passages 1,2,4-60 WO 98 45328 A (CORIXA CORPORATION) Χ 15 October 1998 (1998-10-15) page 2, line 7 -page 5, line 22 page 7, line 23 -page 24, line 11; examples 1-4 sequence listing SEQ ID NOs:1, 3-10, 227 WO 97 25426 A (CORIXA CORPORATION) 1,2,4-60 Χ 17 July 1997 (1997-07-17) page 2, line 8 -page 5, line 11 page 7, line 14 -page 23, line 2; example sequence listing SEQ ID NO:1, 3-10, 227 Patent family members are listed in annex. Further documents are listed in the continuation of box C. Χ Special categories of cited documents: "T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the "A" document defining the general state of the art which is not considered to be of particular relevance earlier document but published on or after the international "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such docucitation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or ments, such combination being obvious to a person skilled in the art. document published prior to the international filing date but "&" document member of the same patent family later than the priority date claimed Date of the actual completion of the international search Date of mailing of the International search report 0 8, 11, 00 8 August 2000 Authorized officer Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, MONTERO LOPEZ B. Fax: (+31-70) 340-3016

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	tion) DOCUMENTS CONSIDERED TO BE RELEVANT Citation: of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.			
Category °	Chasses in document, with indication, where appropriate, of the relevant passages	•	nelevarii io cialm No.		
X	WO 97 25431 A (CORIXA CORPORATION) 17 July 1997 (1997-07-17) page 2, line 3 -page 3, line 25 page 4, line 12 -page 17, line 18; examples 1-4 sequence listing SEQ ID NOs:1, 3-10		1,2,4-10		
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INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)
This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. X Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 21, 22, 29-31 34 37-39 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful international Search can be carried out, specifically:
The second of the minimum probability of high particular of a difference of the second
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)
This International Searching Authority found multiple inventions in this international application, as follows:
see additional sheet a few names ascend not forms due to able
As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
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to the second of
No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Claims 1, 2, 4-60 Partially.
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Remark on Protest The additional search fees were accompanied by the applicant's protest.
No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: Partially 1, 2, 4-60

Breast cancer related polypeptide B18Ag1, corresponding polynucleotides comprising SEQ ID NOs:1, 3-10, or 227, and derived oligonucleotides; variants thereof, expression vector and host cell comprising the same; antibody and diagnostic kit containing it, fusion protein comprising the polypeptide; pharmaceutical composition and vaccine comprising any of the above and use therefor in the treatment of cancer, and for removing tumor cells from a sample; use of the polypeptides for stimulating and expanding T-cells and use of such T-cells for inhibiting cancer development; use of the polypeptides for determining the presence of cancer or monitoring the progression of cancer in a patient.

2. Claims: Partially 1-60

Idem as subject 1 for Breast cancer related polypeptide and polynucleotide B21GT2 (B311D) comprising SEQ ID NOs:56, 307, 308, 316 or 317.

3. Claims: Partially 1, 2, 4-60

Idem as subject 1 for Breast cancer related polypeptide and polynucleotide B15Ag1 comprising SEQ ID NOs:27 or 290.

4. Claims: Partially 1, 2, 4-60

Idem as subject 1 for Breast cancer related polypeptide and polynucleotide B31GA1b comprising SEQ ID NOs:148.

5. Claims: Partially 1, 2, 4-60

Idem as subject 1 for Breast cancer related polypeptide and polynucleotide B38GA2a comprising SEQ ID NOs:157.

6. Claims: Partially 1-60

Idem as subject 1 for Breast cancer related polypeptide and polynucleotide B11Ag1 (B305D) and its isoform A comprising SEQ ID NO:292-306, or 309-315.

7. Claims: Claims: Partially 1, 2, 4-60, all as far as applicable

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Breast cancer related polypeptides, corresponding polynucleotides comprising SEQ ID NOs:11-26 (inventions 7-22), 28-55 (inventions 23-50), 57-86 (inventions 51-80), 142-147 (inventions 81-86), 149-156 (inventions 87-94), 158-226 (inventions 95-163), 228-253 (inventions 164-189), or 255-291 (inventions 190-226), and derived oligonucleotides; variants thereof, expression vector and host cell comprising the same; antibody and diagnostic kit containing it, fusion protein comprising the polypeptide; pharmaceutical composition and vaccine comprising any of the above and use therefor in the treatment of cancer, and for removing tumor cells from a sample; use of the polypeptides for stimulating and expanding T-cells and use of such T-cells for inhibiting cancer development; use of the polypeptides for inhibiting or monitoring the progression of cancer in a patient, as far as applicable.

 $D_{\rm eff}$

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. ational Application No PCT/US 00/09312

	nt documen search rep		Publication date		nt family nber(s)		Publication date
WO 9	845328	A	15-10-1998	EP NO PL	6956098 A 0975666 A 994932 A 336349 A 9802968 A		30-10-1998 02-02-2000 07-12-1999 19-06-2000 27-10-1998
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1	725431	Α	17-07-1997	AU	1575697 A		01-08-1997
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From the INTERNATIONAL SEARCHING AUTHORITY

То:	101
SEED INTELLECTUAL PROPERTY LAW	AUGTATION TO SAVARDETONAL SEES
GROUP PLIC	INVITATION TO PAY ADDITIONAL FEES
Attn. Potter, Jane E.R. ECEIVE	(DCT Adiolo 17/0)(a) and Dula 40.4)
Suite 6300 701 Fifth Avenue CCD 1.9 2800	(PCT Article 17(3)(a) and Rule 40.1)
701 Fifth Avenue FEB 1 2 2002 Seattle, WA 98104-7092	
UNITED STATES OF AMERICA	ly.
SEED INTELLECTION IT AND GO	
· · · · · · · · · · · · · · · · · · ·	Date of mailing (day/month/year) 01/02/2002
Applicant's or agent's file reference	PAYMENT DUE within 45 XXXXXXS/days
210121.41930	within 45 XXXXXS/days from the above date of mailing
International application No.	International filing date
PCT/US 01/16776	(day/month/year) 22/05/2001
Applicant	
CORIXA CORPORATION	
721)	
This International Searching Authority	
(i) considers that there are	umber of) inventions claimed in the international application covered
and it considers that the international application does no	ot comply with the requirements of unity of invention
(Rules 13.1, 13.2 and 13.3) for the reasons indicated/060	OW/on the extra sheet:
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	r
(ii) X has carried out a partial international search (see A	,
on those parts of the international application which relate	e to the invention first mentioned in claims Nos.:
1-17 partially	
(iii) will establish the international search report on the other to which, additional fees are paid	parts of the international application only if, and to the extent
2. The applicant is hereby invited, within the time limit indicated	above, to pay the amount indicated below:
EUR 945,00 x 254	
Fee per additional invention number of additional in	nventions total amount of additional fees
Or, x	=
The applicant is informed that, according to Rule 40.2(c), the pi.e., a reasoned statement to the effect that the international aport hat the amount of the required additional fee is excessive.	payment of any additional fee may be made under protest, oplication complies with the requirement of unity of invention
3. X Claim(s) Nos. <u>further info</u> Article 17(2)(b) because of defects under Article 17(2)(a)	have been found to be unsearchable under and therefore have not been included with any invention.
Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2	Honriotto Hymning Colley
NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,	Henriette Huysing-Solles
Fax: (+31-70) 340-3016	

- 1. The present communication is an Annex to the invitation to pay additional fees (Form PCT/ISA/206). It shows the results of the international search established on the parts of the international application which relate to the invention first mentioned in claims Nos.:
- see 'Invitation to pay additional fees' 2. This communication is not the international search report which will be established according to Article 18 and Rule 43.
- 3.If the applicant does not pay any additional search fees, the information appearing in this communication will be considered as the result of the international search and will be included as such in the international search report.
- 4.If the applicant pays additional fees, the international search report will contain both the information appearing in this communication and the results of the international search on other parts of the international application for which such fees will have been paid.

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 25431 A (CORIXA CORP) 17 July 1997 (1997-07-17) see SEQ ID NO: the whole document	1-17
X	WO 97 25426 A (CORIXA CORP) 17 July 1997 (1997-07-17) see SEQ ID NO: 1 and 2 (pp.34-37) page 1 -page 23; claims 1-42; figures 1,4,6; examples 1-4	1-17
X	WO 98 45328 A (CORIXA CORP) 15 October 1998 (1998-10-15) see SEQ ID NO: 1 and 2 (pp. 34-36) page 1 -page 33; claims 1-49; figures 1,4,6; examples 1-4	1-17
P,X, L	WO 00 61753 A (CORIXA CORP) 19 October 2000 (2000-10-19) L: priority page 1 -page 33; claims 1-60; examples 1-4	1-17

Special categories of cited documents:

[&]quot;A" document defining the general state of theart which is not considered to be of particular relevance

[&]quot;E" earlier document but published on or after theinternational filling date

[&]quot;L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

[&]quot;O" document referring to an oral disclosure, use, exhibition or other means

P" document published prior to the internationalfiling date but later than the priority date claimed

[&]quot;T" later document published after theinternational filing date or priority date and not in conflict with theapplication but cited to understand the principle or theory underlying the invention

[&]quot;X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

[&]quot;Y" document of particular relevance; the claimedinvention cannot be considered to involve an inventive step when the document is combined with one or more othersuch docu ments, such combination being obvious to aperson skilled in the art.

[&]quot;&" document member of the same patent family

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